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*Blood Pressure, Report of Joint Committee on Mortality of The Association of Life Insurance Medical Directors and The Actuarial Society of America, New York, 1925, pp. 10, 52.

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THE AMERICAN JOURNAL OF THE MEDICAL SCIENCES

JUNE, 1956

## ORIGINAL ARTICLES

# THE RECOVERY OF POLIOMYELITIS VIRUSES FROM FECAL SAMPLES OF POLIOMYELITIS PATIENTS AND HOUSEHOLD CONTACTS*

By C. Arden Miller, M.D.
MARKLE SCHOLAR IN MEDICAL SCIENCE

AND

Paul Kamitsuka

(From the Hixon Memorial Laboratory and the Department of Pediatrics, University of Kansas School of Medicine, Kansas City, Kansas)

During the 1954 field trial of the poliomyelitis vaccine the Virus Research Laboratory at the University of Kansas participated in the evaluation of the vaccine, testing more than 243 stool specimens from patients and household contacts for the presence of poliomyelitis virus. The Vaccine Evaluation Center (VEC) had requested that studies be done on first, second, and third grade children who resided in the study areas and who developed clinical evidence of poliomyelitis. There were 97 children studied on this basis. Additional studies on household contacts were undertaken in cooperation with the state and county health officers.**

This report summarizes our data from the VEC program on the recovery of poliomyelitis virus from fecal specimens and correlates these findings with the epidemiologic reports, including those from 35 household units.

**Material and Methods.** The study areas in Kansas, Missouri, and Oklahoma were part of the "observed control study," that is, vaccine was given only to children in the second grade. Children in the first and third grades were observed in order to compare their experience with that of the vaccinated second graders. No placebo was used, making it a simple matter to determine which children had been vaccinated. The methods by which clinically diagnosed and suspected cases of poliomyelitis were identified, their epidemiologic data recorded, and stool and blood specimens obtained have been described in

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**We express our grateful appreciation to Dr. T. R. Hood and Dr. P. A. Bearg of the Kansas State Health Department; Dr. H. S. Albaugh, Dr. M. L. Bauman, and Dr. C. H. Murphy of the Johnson, Sedgwick, and Shawnee County Health Departments (Kansas); Dr. J. R. Amos, and Dr. E. A. Belden of the Missouri State Health Department; Mr. L. J. Miller, Dr. G. A. Harwell, Mr. E. A. Frentzel, Dr. C. G. Leitch, Dr. S. B. Beecher, and Dr. H. L. Dwyer of the Boone, Clay, Greene, Jackson and Pemiscot County and Kansas City Health Departments (Missouri); and Dr. G. F. Mathews and Dr. J. O. Wails of the Oklahoma State Health Department, and Dr. M. L. Peter, Dr. D. V. Hudson, and Dr. T. P. Haney of the Oklahoma and Tulsa County and Oklahoma City Health Departments (Oklahoma).

detail by the Summary Report of the VEC[3]. The same procedures were followed in obtaining epidemiologic data and specimens from household contacts. Any family having a member diagnosed or suspected of having poliomyelitis, and having a child in the first, second, or third grade was accepted for study. An effort was made to obtain a stool specimen on all household contacts and patients as soon as possible after the presence of poliomyelitis in the family was diagnosed. Epidemiologic data (form FT–6)[3] included careful notation of symptoms occurring in all household members and indication of whether poliomyelitis vaccine or gamma globulin had been given to any of the household members.

VIRUS ISOLATIONS. The techniques used in preparing stools for virus isolations have been described[8]. Each stool specimen was tested in 6 roller tube cultures of trypsin-dispersed monkey kidney cells[11]. Fluid was harvested from all tubes showing a cytopathogenic effect within 6 days. Such fluids were mixed with each of the three standard hyperimmune poliomyelitis antiserums[9] and another passage into monkey kidney roller tube cultures was performed in order to type poliomyelitis virus which might be present. Stool specimens which yielded negative results were inoculated a second time into six more roller tubes, and only if the second passage yielded negative results was the stool recorded as negative.

**Results.** HOUSEHOLD EPIDEMIOLOGY. There were 35 households, averaging 5.5 members each, representing a total of 192 individuals in this study. In each household there was one individual who was clinically diagnosed as having poliomyelitis, and who was reported as such to the Health Department. This person is referred to as the "reported case." An additional 21 household members were recorded in the epidemiologic report forms as having some illness or symptom compatible with poliomyelitis or "minor illness." These are referred to as "symptomatic contacts." All but two of the symptomatic contacts had the onset of their symptoms within 7 days either before or after the onset of symptoms in the reported case. One symptomatic contact had an onset 10 days, and another 21 days after the onset of the reported case.

Among household contacts symptoms occurred with the highest incidence in children 4 and 5 years old (Fig. 1).

The most frequent complaints among the reported individuals were fever, headache, and stiff neck; nearly half of them had some paralysis. The most frequent complaints among the symptomatic contacts were fever, sore throat, and headache; paralysis was not reported (Fig. 2). The absence of paralysis among the symptomatic contacts probably means only that the individuals reported to the local health departments as poliomyelitis were the ones with paralysis.

All of the symptomatic contacts occurred in 9 households, giving those households an illness rate of 30/56 (53.6% of household members were ill). The illness rate for the other 26 households was 26/135 (19.1%). Although the symptomatic contacts tended to be confined to a small number of households, there was not a striking association between frequency of symptoms among household contacts and severity of the reported case. In households with a paralyzed person 16/92 (17.0%) of the contacts had symptoms of illness; in households without paralysis 5/65 (7.7%) of the contacts were symptomatic.

VIRUS ISOLATIONS. There were 227 individuals from whom a stool specimen was obtained within 6 weeks after the first symptom in any family member. This figure includes some individual case studies not included in the 35 family surveys. There were 43 paralytic persons; poliomyelitis virus was recovered from 79.0% of them (Table 1). There were 50 nonparalytic cases, 52.0% of whom had positive stool specimens for poliomyelitis virus. Fifty-seven per cent of the 21 symptomatic household

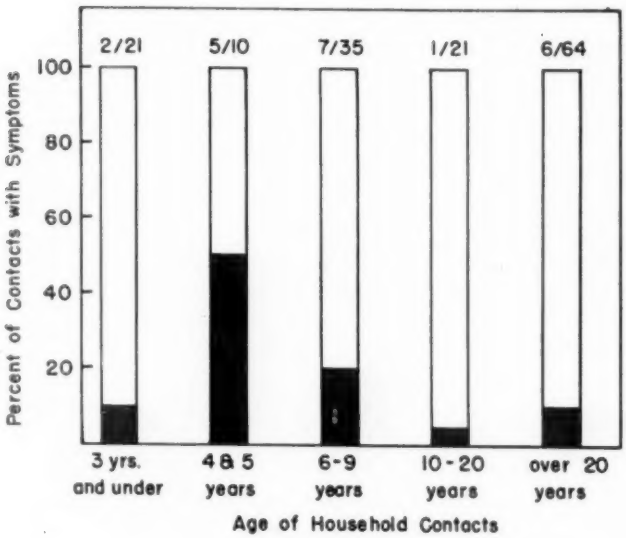


Fig. 1.—Occurrence of symptoms among persons having household contact with clinically diagnosed cases of poliomyelitis.

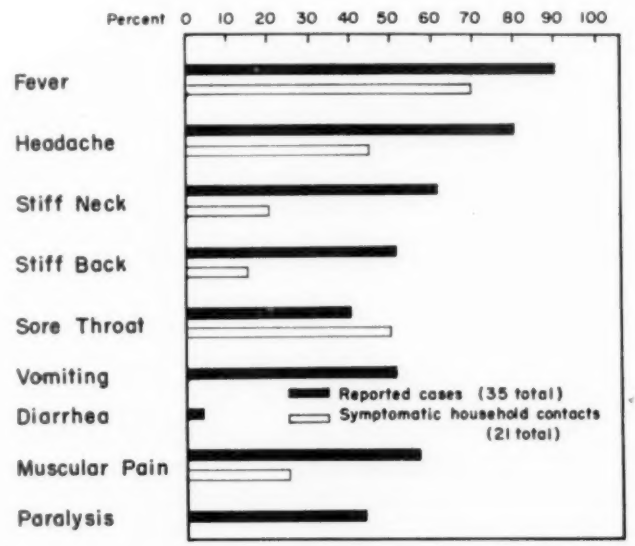


Fig. 2.—Incidence of various symptoms among polio cases and their household contacts.

contacts had poliomyelitis virus; and 34.0% of the 113 asymptomatic household contacts had poliomyelitis virus in their stool specimens (Table 1).

Most stool specimens (82.0%) were collected within 21 days of the first symptoms in any household. Stools collected from individuals 15 to 21 days after onset of symptoms had as high an incidence of poliomyelitis virus as at any earlier time. After 21 days the percentage of positive stool isolations dropped off precipitously, though there were some stools positive for virus even 42 days after onset of symptoms (Fig. 3). The percentage of virus isolations from asymptomatic household contacts showed a striking peak in stool specimens collected 15 to 21 days after the first symptom in any household member. Stool specimens collected from asymptomatic household contacts in the first 7 days after the onset of illness in the household showed a 20.0% incidence of poliomyelitis virus; those collected 15 to 21 days after showed a 57.0% incidence of virus.

The presence of a paralyzed person in a household increased slightly the incidence of virus carriers (Fig. 4).

TABLE 1.—POLIOMYELITIS VIRUS ISOLATIONS

| *Category* | *Positive** | | | *Total Positive* | *Negative* | *Total* |
|---|---|---|---|---|---|---|
| | *1* | *2* | *3* | | | |
| Reported cases: | | | | | | |
| Paralytic | 9 | 13 | 12 | 34 (79.1%) | 9 (20.9%) | 43 |
| Nonparalytic | 6 | 11 | 9 | 26 (52.0%) | 24 (48.0%) | 50 |
| Household studies: | | | | | | |
| Symptomatic household contacts | 8 | 1 | 3 | 12 (57.2%) | 9 (42.8%) | 21 |
| * Asymptomatic household contacts | 17 | 9 | 12 | 38 (33.6%) | 75 (66.4%) | 113 |
| Total | 40 | 34 | 36 | 110 (48.4%) | 117 (51.6%) | 227 |

* Italicized numbers indicate serologic type of poliomyelitis virus recovered.

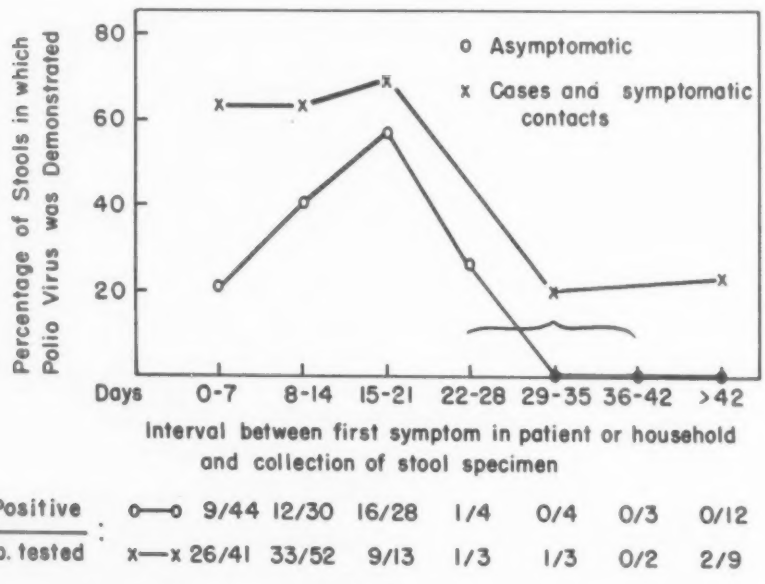


Fig. 3.

The virus carrier rate in households with a paralyzed person was 45.7%; in households without paralyzed individuals, the virus carrier rate was only 32.8%. In households from which at least one member was known to harbor poliomyelitis virus the virus carrier rate was 67/118 (56.7%) (Fig. 4).

symptoms in about the same ratio at all age groups (Table 2). Among household contacts who were virus carriers, symptoms occurred in 18.0% of the persons 10 years of age and over; and in 29.0% of the children under 10 years of age. This difference may be a reflection of the higher incidence of

TABLE 2.—POLIOMYELITIS VIRUS ISOLATIONS FROM HOUSEHOLD CONTACTS

| | *Virus Present* | | *Virus Absent* | | | *Per cent Virus* |
|---|---|---|---|---|---|---|
| *Age Group* | *Symptomatic* | *Asymptomatic* | *Symptomatic* | *Asymptomatic* | *Total* | *Carriers* |
| 3 years and under | 3 | 11 | 0 | 7 | 21 | 66.7 |
| 4 and 5 years | 2 | 5 | 2 | 0 | 9 | 77.8 |
| 6 through 9 years | 5 | 12 | 1 | 10 | 28 | 60.8 |
| 10 through 20 years | 1 | 3 | 0 | 12 | 16 | 25.0 |
| Over 20 years | 1 | 7 | 6 | 46 | 60 | 13.3 |
| Total | 12 | 38 | 9 | 75 | | |

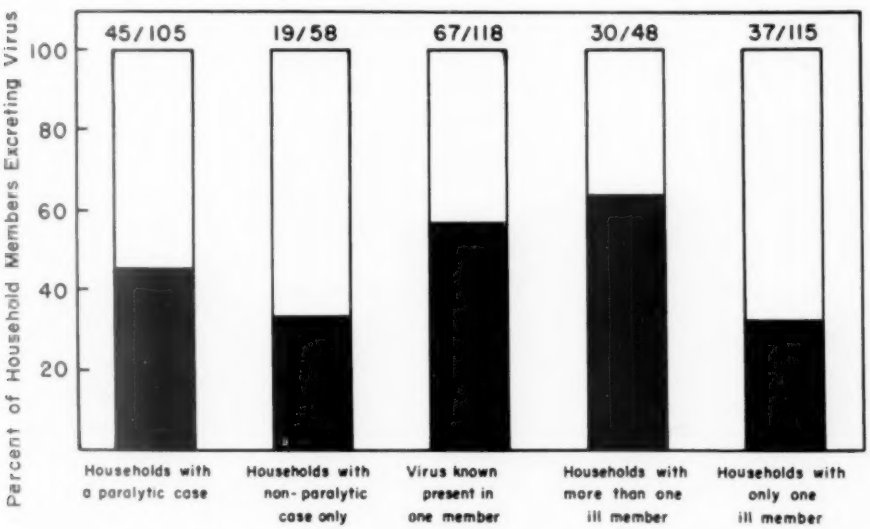


Fig. 4.—Recovery of poliomyelitis virus from household members.

The highest incidence of poliomyelitis virus carriers among household contacts occurred in children under 10 years of age (Table 2). The peak incidence (77.8%) occurred in children 4 and 5 years old.

The virus carrier state among household contacts was associated with symptoms among all younger household contacts, in the virus carrier group as well as in those from whom no virus was recovered.

EXPOSURE TO POLIOMYELITIS VIRUS IN A HOUSEHOLD. There were 21 households in which at least one member was a poliomyelitis virus carrier. The ill-

ness rate among the contacts of these households was 20/101 (19.8%). There were 14 households in which no member was found to harbor poliomyelitis virus. The illness rate among the contacts in these households was 0/55 (0.0%), no symptoms having been recorded for any family members except the reported cases.

HOUSEHOLDS WITH SYMPTOMATIC CONTACTS. Mention has been made that all 21 symptomatic contacts were in 9 households. The virus carrier rate among the members of these 9 households was 30/48 (62.5%). The virus carrier rate among the members of households with only reported cases and asymptomatic contacts was 37/115 (32.2%) (Fig. 4).

OTHER VIRUSES. Viruses other than poliomyelitis were recovered from 6 stool specimens. Three of these viruses were Coxsackie, two of the same type being recovered from different members of the same household; two others were a mixture of Coxsackie and poliomyelitis viruses; and one other virus was not identified. If the tissue cultures had been kept longer than 6 days it is probable that more "orphan viruses" would have been recovered[7].

EFFECT OF GAMMA GLOBULIN. One or more members in 14 of the 35 study households received gamma globulin injections, a total of 42 individuals. The exact amount of gamma globulin given to each person was not always recorded. Only one of the reported cases received gamma globulin; this was given 3 days before onset of paralytic symptoms.

*Symptoms.* The mean interval between the onset of symptoms in the reported case and the injection of gamma globulin into household contacts was 5 days (range 1 to 12). Five contacts had developed symptoms prior to receiving gamma globulin injections; four others, all in the same family, developed symptoms following gamma globulin injections. Excluding the persons who were ill prior to their injection the ratio of symptoms among household contacts receiving gamma globulin was 4/35 (11.4%). The ratio of symptoms among household contacts not receiving gamma globulin was 17/116 (14.6%). Gamma globulin in this small group appeared to have no effect in the development of symptoms among household contacts.

*Poliomyelitis virus carrier state.* Stool specimens were collected from 36 family contacts after they had received gamma globulin. Among these stool specimens there were 17 (47.2%) which yielded poliomyelitis virus. The virus carrier state among household contacts who did not receive gamma globulin was 17/116 (34.0%). The high poliomyelitis virus carrier rate among household contacts receiving gamma globulin may be explained by the younger average age of these persons compared to the total group, and by the higher incidence of exposure to paralytic cases than the total group; both of these factors correlated with an increased poliomyelitis virus carrier rate.

POLIOMYELITIS VACCINE. There were 11 children who were reported to have received one or more poliomyelitis vaccine injections. Because of the small number of cases, and because they came from areas where vaccine of poor antigenicity had been used, they were not analyzed separately.

**Discussion.** Recognition that poliomyelitis may affect several members of a household simultaneously was first made clinically and later amply documented with laboratory evidence[1,10]. With the greater application of laboratory procedures in the isolation of poliomyelitis virus and determination of specific antibody responses, increasing support was found for the view that

poliomyelitis was a "family disease," that when a case of poliomyelitis is diagnosed, all household contacts must be assumed to be already infected, to be incubating infection, or to have a prior immunity. That this view may pertain to some families is certain; but whether it is true universally is not known.

Judging from the occurrence of symptoms alone, more than one case of poliomyelitis occurred in only one-third of the households. Interestingly enough in these households over half the members were ill,. thus approaching the concept of a household infection. However, in the majority of households infection was not clinically apparent except in the one "reported case" per household. There was not an appreciable difference in the incidence of illness in households with paralyzed individuals, versus those without paralysis.

If one considers that the recovery of poliomyelitis virus from stool specimens may give more accurate knowledge of infection than the occurrence of symptoms, then a somewhat different impression of household epidemiology is obtained. In households with a paralyzed individual, 45.7% of the household members were carriers of virus. The incidence of infection was only slightly greater than this in households from which at least one member was shown to be a poliomyelitis virus carrier; the incidence of virus carriers among all these household members was 56.0%. The most striking finding relates to an analysis of the excretion of virus by asymptomatic household contacts at various intervals from the first onset of symptoms in any household member. The percentage of well contacts who excreted virus increased from 20.0% in the first week to 57.0% in the third week. This peak in the third week may reflect a substantial portion of household contacts who became infected from the first case in the household, rather than from simultaneous exposure of all household members by an outside source.

The assumption that the presence of poliomyelitis virus in a stool specimen is the best evidence of poliomyelitis infection cannot go unchallenged. An associated rise in specific serum antibody titer would be more convincing evidence. Unfortunately, with present techniques this evidence is not at hand. The difficulties in establishing the serologic criteria of poliomyelitis infection are presented in another paper[6].

The data presented here on the recovery of viruses by tissue culture techniques invites comparison with a similar study by Horstmann, Ward, and Melnick[5] using intracerebral inoculation of monkeys. The percentage of virus isolations from paralytic patients was increased from 62.0% in monkeys to 79.0% in tissue cultures. Godenne and Riordan[4], using tissue culture techniques, report an even higher recovery rate (89.0%) of poliomyelitis virus from the stools of paralyzed patients. From nonparalytic patients Horstmann reported 77.0% positive isolations; in our series only 52.0% of nonparalytic patients yielded virus. In view of the apparent greater efficiency of the tissue culture method[8] the discrepancy in results from nonparalytic patients is difficult to explain except by the suggestion that agents other than poliomyelitis virus were responsible for some of the nonparalytic cases in our study.

Data are available from Horstmann's study as well as ours on the percentage of patients excreting poliomyelitis viruses at various intervals during convalescence. There is agreement between the two studies that virus can be recovered from a majority of the stool specimens in the first weeks of convalescence. In our study the percentage

of positive isolations dropped sharply to 20.0% in the fourth and sixth weeks; virus isolations from Horstmann's study did not drop to this level until after this time. This difference may be accounted for by assuming that the quantity of virus excreted in the stool decreases after the first week. The smaller number of viruses perhaps can be demonstrated by testing the pellets obtained from ultracentrifugation of the stool specimens. This was done by Horstmann. In our study the stools were tested after light centrifugation, and it is likely that the concentration of virus in some stool specimens was too low to cause a cytopathogenic effect in the tissue cultures.

The virus carrier rate among household contacts was highest in children 4 and 5 years old, and remained at high levels until 10 years of age, when presumably as a result of prior immunity, the virus carrier rate dropped rather precipitously. Children, 4 and 5 years old, who were in contact with an infected person also had the highest incidence of symptoms. The peak incidence of poliomyelitis infections at this age is difficult to explain; from serologic evidence, children are susceptible to infection at an earlier age. Possibly children, 4 to 5 years old, represent in our society an age group no longer protected by maternal antibodies and by sheltering from intimate exposure, and not yet protected by immunity due to prior infection.

Of further interest is the fact that about 20.0% of the household contacts who were shown to be carriers of poliomyelitis virus were recorded as having symptoms. If one can assume that the presence of poliomyelitis virus in a stool specimen is evidence of infection, then 80% of all such infections within the family unity can be assumed to be clinically "silent."

Brown has reported that the administration of gamma globulin to household contacts does not interfere with the demonstration of poliomyelitis virus in their stool specimens[2]. Our observations confirm this finding.

**Summary and Conclusion.** From a study of the epidemiologic and laboratory data from nearly 250 poliomyelitis patients and household contacts the following information was derived.

1. Family infections due to poliomyelitis were clinically apparent in about one-third of the families studied. Symptoms in the different members of a family tended to occur concurrently. Children, 4 and 5 years old, who were in contact with a known case of poliomyelitis were most apt (50.0% of those exposed) to develop a clinically apparent illness.

2. Seventy-nine per cent of paralyzed individuals yielded poliomyelitis virus in their stool specimens. Only half of the nonparalyzed persons yielded poliomyelitis virus, perhaps indicating that a number of these infections were caused by some agent other than known poliomyelitis viruses. One-third of all persons having household contact with a clinically diagnosed case of poliomyelitis were shown to have poliomyelitis virus in their stool specimens. The virus carrier rate was much greater than this, a) among household contacts who had some symptom indicative of illness, b) among those who were exposed to a paralytic patient, and c) among those under 10 years of age.

3. Excretion of virus in fecal samples was maintained at a high level for the first 3 weeks of convalescence, after which the recovery rate dropped sharply. The percentage of asymptomatic household contacts excreting virus showed a decided peak 3 weeks after the first onset of symptoms in the household.

4. Among the household contacts

80.0% of the virus carriers were free of symptoms of illness.

5. The administration of gamma globulin to household contacts appeared not to interfere with the demonstration of virus in their fecal specimens.

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## SUMMARIO IN INTERLINGUA

### Le Identification de Virus de Poliomyelitis in le Feces de Patientes Poliomyelitic e de lor Contactos Familial


Le sequente constatationes esseva establite super le base de un studio del datos epidemiologic e laboratorial ab 250 patientes de poliomyelitis e lor contactos familial.

1. Infectiones familial debite a poliomyelitis esseva clinicamente apparente in circa un tertio del familias studiate. Le symptomas occurrente in varie membros de un familia tendeva a manifestar se contemporarimente. Juveniles de 4 e 5 annos monstrava le plus alte procentage de clinicamente apparente morbos disveloppate post contacto con un caso cognoscite de poliomyelitis. In iste gruppo de etate, tal morbos occurreva in 50 pro cento del individuos exponite a poliomyelitis.

2. In 79 pro cento del individuos paralysate, virus de poliomyelitis esseva trovate in le feces. Solmente un medietate del individuos non-paralysate habeva virus de poliomyelitis in lor feces. Isto indica possibilemente que un certe numero del infectiones non-paralysante esseva causate per agentes altere que le cognoscite viruses de poliomyelitis. Un tertio de omne personas in contacto familial con clinicamente diagnosticate casos de poliomyelitis habeva demonstrate virus poliomyelitic in lor feces. Un multo plus alte procentage de portatores de virus poliomyelitic esseva trovate inter (a) contactos familial con symptomas de morbo, (b) contactos exponite a patientes paralytic, e (c) contactos de etates de infra 10 annos.

3. Le excretion de virus in le feces se manteneva a un alte nivello durante le prime tres septimanas del convalescentia. Postea le numero del identificationes del virus in le feces se diminueva acutemente. Le procentage de asymptomatic contactos familial qui excerneva virus in lor feces attingeva un culmine pronunciate 3 septimanas post le prime declaration de symptomas in le familia.

4. Inter le contactos familial, 80 pro cento del portatores de virus esseva libere de symptomas clinic.

5. Il pareva que le administration de globulina gamma a contactos familial habeva nulle effecto super le demonstration de virus in specimens fecal.

# QUANTITATION OF THE ACTIVITY OF RHEUMATOID ARTHRITIS

## I. A METHOD FOR RECORDING ITS SYSTEMIC MANIFESTATIONS

By John Lansbury, M.D.
PROFESSOR OF CLINICAL MEDICINE

(From the Department of Medicine, Temple University School of Medicine, Philadelphia, Pennsylvania)

Physicians studying rheumatoid arthritis have long been hampered by the lack of dependable criteria for measuring the degree of activity of this disease. Most have tried to estimate activity from the appearance of the joints and the erythrocyte sedimentation rate only. Systemic manifestations of the disease have been neglected as a "yardstick" for measuring activity because many of them are too subjective, that is, manifested by symptoms rather than physical signs, and, therefore, defying quantitation. The object of this paper is to show how such systemic manifestations have proved valuable in clinical quantitation of the degee of rheumatoid activity.

THE CONCEPT OF RHEUMATOID ACTIVITY. Clinicians and pathologists alike agree that rheumatoid arthritis is not, as its name implies, a disease of joints alone. Rather, it is a systemic disease often involving the central nervous system, the vasculature, the whole connective-tissue system (including muscles, bones, ligaments, cartilages, tendons and their sheaths, fascia, and others) as well as the hematologic and immunity mechanisms and even the lungs. Clearly, such widespread devastation deserves to be taken into account in any scheme of evaluation. The problem is to select from this confusing plethora of manifestations those few items which are a reliable guide to the activity of the disease.

What then are we really trying to measure? To answer this question we must again allude to the nature of the disease. Because this is quite unknown, we are forced to measure the "activity" of the process by its *inflammatory manifestations*. These may be likened to a smouldering fire which slowly consumes the body's supporting structures and ultimately leaves behind a mass of twisted wreckage which we refer to as deformities. The ultimate aim of all medical therapy is to dampen or to put out the fire. The ultimate aim of all methods of evaluation is to measure the amount of this smouldering inflammation. The technique of *joint* evaluation is concerned with the effects of the inflammatory process on the joints and their attending structures. The technique of *systemic* evaluation is concerned with measuring the body's total inflammatory response. Both are valid methods. Both give some information as to the activity of the inflammatory process.

THE PROBLEM OF RECORDING RHEUMATOID ACTIVITY. With the nature of "rheumatoid activity" thus defined, we are now in a position to mention briefly some problems which face the doctor at each office visit when he has to record some meaningful statement as to "how the patient is doing."

The patient himself is only too willing to solve the problem by making such statements as: "I am about the same," "I feel much worse" or "I feel a lot better." At the time such statements are made they may reflect with reasonable accuracy any change which

may have occurred since the time of the last visit, and the busy doctor too often succumbs to the temptation to incorporate such statements into his record to the exclusion of really informative data. A case record which consists preponderantly of such statements is unfortunately utterly worthless as a guide to what has really been going on. What does "feeling better" or "feeling worse" mean a year later? To be meaningful we should at least have stated what it was that was better or worse, and how much. In other words, such generalities, even though made by the doctor as a record of his professional evaluation, are valueless in retrospect because they cannot be referred to a known, standard base-line.

JOINT EVALUATION AS A MEASURE OF ACTIVITY. Joint evaluation has been, until now, the main remedy for this unsatisfactory state of affairs. There can be no doubt that an expert examination of each affected joint as to range of motion, swelling, fluid, tenderness, heat and deformity does furnish accurate, objective information as to the course of the disease, insofar as it relates to the joints themselves; but it gives very little information as to the general status of the patient. Furthermore, it often fails to distinguish between present (active) disease and the effects of old (extinct) disease; it demands a high degree of skill for which special training or long experience are necessary, and it is so time-consuming that it is often impossible to perform in the time allotted for an office visit.

Let us suppose that a given patient has 10 of his 60-or-so examinable peripheral joints involved by the rheumatoid process. If each joint is to be observed as to heat, tenderness, swelling, fluid, limitation of motion and deformity, and, if the degree of each of these is noted in grades of I to IV, then the examiner has over 200 decisions to make and record within the time of an office visit! It is no wonder that the doctor feels swamped with data and is forced to confine his observations to a few "key" joints or to resort to the vague, general statements referred to above.

We do not imply that joint evaluation is not valuable. On the contrary, it is indispensable. But, because of its limitations, it should perhaps only be used at long intervals, such as at the beginning and at the end of a course of treatment, or every 3 to 6 months. The weekly or monthly visits may then be devoted to the 4 or 5-minute procedure of recording the systemic manifestations as will be discussed below.

THE OBJECTIVE, SYSTEMIC MANIFESTATIONS OF RHEUMATOID ARTHRITIS. The following items objectively measure the systemic manifestations of rheumatoid arthritis: 1) fever, 2) weight loss, 3) increased ESR, 4) anemia, 5) reversed A/G ratio, and 6) C-reactive protein. Although none of these is a specific manifestation of rheumatoid arthritis, each is a valid indicator of "activity." Their individual values in relation to total activity must be determined by further study.

It seems self-evident that a patient whose systemic reaction to rheumatoid arthritis is reflected by an oral temperature of 103° F. is manifesting a greater degree of rheumatoid activity than is a similar patient whose temperature is normal. Likewise, a patient who is losing weight (other causes being excluded) is sicker than one who is not. There is general agreement that the sedimentation rate reflects, within certain limits, the general or systemic activity of the disease. Anemia, if not previously present and if not due to other, unrelated causes, is likewise a systemic feature of the disease, and is reversed when the patient goes into a remission. Alterations in serum albu-

min and globulin, often a serious prognostic omen, are nevertheless capable of returning to normal if a patient attains a true remission. The C-reactive protein determination appears to have a useful function as an indicator of activity in rheumatic fever, and preliminary studies suggest that it may also be a guide to the total inflammatory response in rheumatoid arthritis.

QUANTITATION OF SYMPTOMATOLOGY. As mentioned above, the symptoms of rheumatoid arthritis have been considered as being too subjective to enter into any scheme of evaluation. All clinicians know that a sensitive patient may describe a given pain as "severe" or "terrible," whereas a phlegmatic patient may dismiss what is apparently the same degree of pain as being merely "bothersome." Any statement, therefore, as to the severity of rheumatic symptoms is next to impossible to evaluate. How then can we objectively evaluate a subjective symptom?

The difficulty disappears as soon as we give up the idea of guessing at the *degree of severity*, and instead, measure an objective feature, namely the *duration* of the symptom. It so happens that nearly all the symptoms of rheumatoid arthritis have a certain diurnal periodicity. They can therefore be objectively quantitated by recording the *number of hours* during which they persist each day. (Unfortunately, motion pain is probably an exception to this rule.)

Let us now see how the yardstick of duration may be applied in evaluating the following arthritic symptoms: 1) pain at rest, diurnal, vesperal, nocturnal, 2) pain on motion, 3) morning stiffness, 4) jelling (diurnal stiffness after rest), 5) fatigue, 6) salicylate consumption, and 7) weakness.

*1) Rest Pain.* A clear distinction can usually be made between pain which is present at rest and pain which is experienced only on movement of a joint. Rest pain is usually of an aching or throbbing type. It probably indicates the most severe phase of articular inflammation. It is not very often seen in ambulatory rheumatoid patients, but when present can be measured by its duration in hours per day. It is to be distinguished from evening aching which may result from prolonged overuse of inflamed joints and which is more commonly seen in osteoarthritis. Nocturnal rest pain may also be distinguished from pain which wakes the patient as he moves in his sleep, and from the early morning stiffness which may wake him at dawn. In general, it is difficult to get accurate information about night symptoms, since the patient is generally drowsy. However, some mention of night pain and stiffness is worthwhile because nocturnal symptoms are often seen in the more severe phases of the disease and frequently disappear early in the course of an oncoming remission.

*2) Motion Pain.* The sharp pain in the joints which occurs on attempted movement is one of the first symptoms to appear in the ordinary course of rheumatoid arthritis and one of the last symptoms to disappear as the patient goes into a remission. Unfortunately it does not lend itself to measurement by duration in hours per day; so, for the present we simply record it as present or absent. We have tried for a year to record it as absent, slight, moderate or severe, but our over-all impression is that this cannot be reliably done. We are exploring the possibility of estimating it in terms of total joint surface involved.

*3) Morning Stiffness.* This is one of the most constant symptoms of rheumatoid arthritis. It may be defined as a resistance on the part of affected joints to motion on awakening in the morning. Yet, it is not a specific manifestation of the disease since it may be seen after

unaccustomed athletic activity, in primary fibrositis and in other forms of "rheumatism."

In recording its duration, one ascertains the time at which the patient rises and the hour at which the stiffness wears off. We have been asking this question for over 10 years and we find that the majority of patients can give an accurate account of the duration of morning stiffness *if the question is put in this way*. The doctor must compute the number of hours or fractions thereof during which the symptoms persist. If the patient is simply asked "how long are you stiff in the morning" he will often give a vague guess.

An analysis of our records of duration of morning stiffness shows that when averaged for a group of patients going into a remission, the hours of morning stiffness recede at a regular rate with the declining activity of the disease. We regard duration of morning stiffness as a reliable quantitative index of the activity of the disease, provided the first dose of aspirin has been constant in amount and taken at the same time.

*4) Diurnal Jelling.* This may be considered as "morning stiffness in miniature." It consists of stiffness which occurs during the day after the patient has been sitting or lying down for varying periods of time. It probably cannot be quantitated because it depends on the number of times the patient rests during the day and the length of time he rests. Insofar as we can see, its severity seems to parallel that of morning stiffness. For purposes of recording, it can be noted as present or absent, and serves to check the patient's statement as to morning stiffness.

*5) Amount of Salicylates Used Per Day.* There is clearly a great difference between the state of a patient who requires 16 aspirin tablets per day to control his pain and a patient who needs only 2 aspirin tablets per week to control his symptoms; but it is doubtful if an "aspirin tablet count" is sensitive enough to be used as an index of activity. Nevertheless, it is worthwhile recording, since it confirms the amount of pain which may be present.

*6) Fatigue.* This can be estimated in ambulatory patients by ascertaining how many hours of ordinary occupation they can perform before feeling the need to rest. It must be distinguished from a neurotic lack of desire to do anything. Severely ill patients feel fatigued even at rest. Ambulatory patients may be able to work one to 8 or even more hours without feeling tired. We have found this estimate of the duration of normal energy output to be a promising item for quantitative evaluation, but we have not as yet observed its course in patients going into remission, and therefore cannot make a statement as to its quantitative validity. For the present is seems worthwhile to record it as a measure of systemic activity.

*7) Weakness.* Muscle weakness may exist apart from joint inflammation; yet it is extraordinarily difficult to measure as pure weakness. It is too often "contaminated" by joint pain and deformity. Nevertheless, measurements of the strength of the grips by compression of an inflated blood-pressure cuff or by a hand dynamometer, and measurements of lower extremity strength by timing the ascent and descent of a standard flight of steps, provide valuable information and may well be incorporated into the simple scheme which we propose below, although they are not strictly systemic manifestations. While "duration" does not enter into these determinations, there may be considerable diurnal variation in muscle strength so that the measurements should be made at the same time of day on each occasion.

A FORM FOR RECORDING SYSTEMIC MANIFESTATIONS. We have used the following form (slightly modified) for collecting pertinent data on the systemic manifestations of rheumatoid arthritis for approximately one year (Fig. 1). The form is stamped on any convenient part of the patient's chart by an ordinary rubber stamp on the occasion of each visit.

Items 1 to 6 constitute a miniature history which, once the patient is used to the procedure, requires only 3 to 5 minutes to elicit and record. Items 7 to 11, inclusive, record general physical findings which, together with the laboratory findings, (items 12 to 16, inclusive) can be recorded by an office secretary or nurse and therefore make no demand on the doctor's time. The laboratory determinations may have to be performed less frequently than the purely clinical observations because of their expense to the patient. For purposes of clinical research, such as testing the response to a new drug, all items should, if possible, be recorded.

(1) Rest pain: (Hrs.) Day____ Night____ Eve____.
(2) Motion pain: None ( ) Slight ( ) Severe ( )
(3) Morning stiffness: Hours ( ) Minutes ( )
(4) Diurnal Jelling: Present ( ) Absent ( )
(5) Fatigue: None ( ); after ( ) hrs. normal work.
(6) # ASA tablets / day to control pain ( )
(7) 4 P.M. Temperature ( )
(8) Basal Body Weight ( )
(9) Grips: R____ L____
(10) Steps Up____ Down____
(11) Rises from chair in ( ) seconds ♂ ( ) ♀ ( ) aid.

(12) Gm. Hb____ (13) ESR____ (14) S.Alb.____ (15) S.Glob.____ (16) C-reactive Prot.____

Fig. 1.—Rubber-stamp form for recording systemic manifestations in rheumatoid arthritis.

We have found this form to be practicable and informative since the data collected over long periods of time can be compared. Evaluation of activity by any method is, of course, at its best in early cases, irrespective of their severity, since in such cases the clinical picture is not blurred by deformities. Moreover, it is in early cases that the question of activity is of paramount importance.

It seems clear that if *all* the above findings are *really* negative, that the patient is most unlikely to have a significant degree of systemic rheumatoid activity. Any residual disability which he may have will therefore be most likely due to deformities caused by *past* activity and will need orthopedic and physical therapy rather than medical treatment.

While a complete clinical remission can easily be detected by the above data, we are not as yet sure of the place of each item as an indicator of grades of activity. When this has been determined by observing a sufficient number of patients going into a complete remission, we hope to be able to evaluate each item mathematically and to express the percentage activity of the whole by a number.

Since complete remissions are rather uncommon, it is hoped that rheumatologists and others interested in the rheumatic diseases will try out the above method for themselves and thus determine the value of each item. In its present stage of development we believe that the above form can be of use to all doctors endeavoring to assay the degree of activity in their rheumatoid patients. Because any measure of rheumatoid activity must depend on an observable measure of both systemic and articular inflammation, this (or any other) method cannot measure the course of the disease when its inflammatory manifestations are being

suppressed by steroid therapy. In such cases it may measure the anti-inflammatory action of a given drug, but *not* the underlying amount of rheumatoid activity.

**Summary and Conclusions.** 1) The activity of rheumatoid arthritis is gauged by the degree of the body's inflammatory response. This response may be systemic or articular, or both. Joint manifestations are only one facet of the disease picture.

2) Until now, the methods for evaluating rheumatoid activity have been centered on examination of the joints, and the systemic manifestations of the disease have been neglected.

3) A quick and apparently reliable method for recording systemic manifestations of the disease is presented which includes a method for objectively evaluating subjective symptoms by measuring their duration rather than their intensity.

4) The evaluation of the systemic manifestations of the disease is recommended as an adjunct to joint evaluation in arriving at a reliable estimate of the status of the patient.


## SUMMARIO IN INTERLINGUA

### Quantitation del Activitate de Arthritis Rheumatoide
### 1. Un Methodo pro le Registration de su Manifestationes Systemic

1. Le activitate de arthritis rheumatoide es reflectite in le grado del responsa inflammatori del corpore. Iste responsa pote esser systemic o articular o ambe. Manifestationes articular es solmente un del aspectos del syndrome.

2. Usque nunc, le methodos de evalutation del activitate rheumatoide esseva preoccupate con examines del articulationes. Le manifestationes systemic del morbo esseva negligite.

3. Es presentate un rapide e apparentemente exacte methodo de registrar le manifestationes systemic del morbo. Illo include un evalutation objective de symptomas subjective, basate super le mesuration de lor duration plus tosto que de lor intensitate.

4. Le evalutation del manifestationes systemic del morbo es recommendate como adjuncto al evalutation articular in le formulation de un solide judicamento in re le stato del patiente.

# OBSERVATIONS ON CERTAIN VIRUSES CAUSING EXANTHEMATOUS DISEASES IN MAN*

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In this lecture I propose to review certain observations especially in respect to etiology, which my associates and I have made on three exanthematous diseases. All these conditions are usually benign. Two of them, among the minor plagues of mankind, are old acquaintances, the third has only recently emerged as a clinical and epidemiologic entity.

Many in this audience, will, I feel sure, wonder why anyone should spend much time in reinvestigating measles, chickenpox and a mild infection which, for lack of a happier epithet, we have so far referred to as "an unusual epidemic exanthem," when other conditions of a more serious or dramatic character remain to be explained and controlled. Our reasons for so doing are several. With little doubt, the underlying emotional impetus lay in the challenge presented by the fact that in spite of many endeavors by workers in the past no satisfactory ways had been found to isolate and maintain the agents of measles and chickenpox in the laboratory. We were also impelled by motives of a more purely scientific character. Thus, if it should prove possible to discover means whereby these viruses could be propagated at will, a systematic study of their properties might be carried out. The results would almost certainly serve to extend in a profitable manner that body of rapidly increasing knowledge which is now designated by the somewhat unlovely name of "virology." In turn, the acquisition of such information might lead to the development of methods of diagnosis and control.

Exploration of the exanthematous disease that I have just mentioned has been accomplished almost entirely by my former associate, Dr. Franklin Neva. It was undertaken opportunistically because this infection manifested itself in the form of a widespread epidemic in Massachusetts during the summer of 1951. Since the physicians were unable to classify it among any of the known rash diseases, it obviously became a matter of much interest to define, if possible, the responsible agent.

Because the tissue culture enabled us in a large part to accumulate the data which I am about to review, I shall by way of additional introductory matter comment briefly on this valuable method. Although the procedure has been employed on occasion to the study of viruses by many investigators during a period of over forty years, its potentiality did not become fully apparent until quite recently. Three circumstances at least may be cited to account for the relatively meager harvest of results gathered in the past by means of the tissue culture.

The first of these was the failure to distinguish constant and reliable criteria for the multiplication of the virus within the culture system itself, analo-

*The Gordon Wilson Lecture of the American Clinical and Climatological Association delivered at the Annual Meeting, October 31, 1955.

gous to the manifestations of the disease in experimental animals. To determine, therefore, whether or not virus had increased it was always necessary to inject susceptible animals with material from the culture. Accordingly for most purposes, cultivation *in vitro* of the virus appeared to offer no advantages over direct animal inoculation.

In this respect the shortcomings of the tissue culture have in many instances been overcome by demonstration of the fact that the majority of viruses so far examined produce, as they multiply, degenerative changes in the cells. These changes are often apparent within a few hours or a few days after the viral inoculum is introduced and frequently are sufficiently characteristic to permit a tentative identification of the virus under examination. Such effects are now referred to as "cytopathic changes" or as "cytopathogenic effects of the virus."

Although cytopathic changes were noted earlier by various investigators, especially by Ivanovics and Hyde[6] in 1932 and Huang[5] in 1942, their full significance was not generally appreciated until 1950, when it was demonstrated (Robbins, Enders and Weller[13]) that the viruses of poliomyelitis were highly cytopathogenic in cultures of a variety of several human tissues. Since that time results obtained with a large number of viruses and a variety of cells from various species show that exhibition of cytopathogenicity *in vitro* affords criteria of viral multiplication as reliable as the production of signs of infection in animals. Moreover, such criteria may be often more conveniently and accurately observed.

A second circumstance responsible for the earlier unfruitfulness of the method lay, I think, in the lack of adequate appreciation of the fact that multiplication of certain viruses depends upon the presence of cells derived from susceptible animals. Previously it was unclear whether or not the natural host-virus susceptibility relationship was maintained in isolated cell systems. Numerous workers, therefore, continued to employ tissues that were most easily procured in the laboratory such as those of the chick embryo or the rabbit's testicle in unsuccessful attempts to cultivate viruses like those of poliomyelitis or chickenpox which are characterized by a high degree of specificity both *in vivo* and *in vitro*. The isolation of the new group of respiratory viruses first described by Rowe and his associates[15] as well as the so-called "orphan," "human enteric viruses," or "ECHO viruses"* representatives of which were first obtained in our laboratory[14,19] and have been subsequently and extensively studied by Melnick[7], Sabin[12] and others rests in part upon the recognition of this fact.

Just as the growth of virus may occur only in the presence of cells from a susceptible species, so multiplication may take place only in a given type of cells such as fibroblasts or epithelial cell elements derived from that species. Although in the earlier literature there are to be found a few examples of such cytotropism *in vitro*, recent observations have stressed its importance in the selection of media for the cultivation of certain viruses.

A third factor and perhaps the most decisive of all in formerly limiting the value of the tissue culture was the difficulty in avoiding contamination by various microörganisms. Elaborate precautions had to be taken to prevent the entrance of bacteria which greatly restricted the number of cultures that could be prepared and maintained.

*It has very recently been proposed[2] that viruses having these properties be designated provisionally as "ECHO" viruses (Enteric Cytopathogenic Human Orphan Viruses).

Moreover, it was possible to introduce into the system for examination only materials which had been rendered free of bacteria by filtration, a procedure which no doubt often reduced any virus that might be present to a subinfectious level. When antibiotics became available, this situation was entirely changed. At present, not only can very large numbers of cultures be employed as routine, but also specimens of feces or throat washings may be added with little chance that bacterial growth will follow.

Of late, other advances in technique have been made which increase enormously the usefulness of the method. Most notable, perhaps, are the introduction of cultures of human malignant cells by Scherer, Syverton and Gey[17] and the preparation of normal and malignant cell suspensions by prolonged treatment of the tissue with trypsin. The procedure of trypsinization devised many years ago by Rous and his associates but almost forgotten until recently was re-introduced by Dulbecco and Vogt[3] in 1953. By its means material sufficient for practically unlimited numbers of cultures can be obtained, the use of a plasma matrix avoided and a mass of cell growth developed in which the cytopathic changes induced by virus are admirably revealed.

For two reasons I have run the risk of boring you with this most imperfect sketch of some of the recent technical advances in the art of tissue culture as applied to the cultivation of viruses. In the first place I wanted to make it evident that the method has been developed to a point that renders it almost as indispensable and as versatile in both the theoretical and practical study of viruses as the agar plate and the broth culture in the field of bacteriology. Secondly, I felt that it might serve as useful background for some, enabling them to visualize more precisely the means by which the etiologic investigations were carried out which I mentioned at the outset and which I shall now describe.

INFECTIOUS EXANTHEM. I shall begin with the unusual infectious exanthem. As I have said, we first became aware of this condition during the summer of 1951. A subsequent study of cases of this disease as reported by pediatricians to the Massachusetts Department of Health indicated that it was widespread throughout the state, since about 2,000 probable cases were recorded in this manner (Neva, Feemster and Gorbach[10]). Another but smaller outbreak of the same illness occurred in Pittsburgh in 1954 which was likewise investigated by Dr. Neva[8]. A careful study of a limited number of cases in households both in Boston and Pittsburgh revealed a high communicability and showed that both children and adults were attacked. The incubation period was defined as ranging between 3 to 8 days. In the majority of instances, however, symptoms appeared 4 to 5 days after exposure. The disease was mild, lasting only from 2 to 5 days. Its manifestations were definitely influenced by age. In children, most of whom fell in the 2- to 8-year group, a febrile period of one or two days was either followed by or associated with a pink maculopapular skin eruption over the face, trunk and extremities which persisted for 2 or 3 days. In Fig. 1 the appearance of the rash is illustrated. Systemic signs and symptoms were usually minimal. Occasionally moderate enlargement of the lymph glands of the head and neck was observed but this was by no means a constant feature. In contrast the illness in adults was prominently distinguished by systemic manifestations such as chills and fever, severe headache and muscle pains and aches. In only one-half of the older cases studied was a rash observed. It was frequently

TABLE 1.—SUMMARY OF PRINCIPAL FEATURES IN 16 PATIENTS WITH EPIDEMIC EXANTHEM*

| *Feature* | *No. Patients* | *Data and Remarks* |
|---|---|---|
| | | *Age in Years:* |
| | 2 | 2 |
| Age Distribution | 10 | 2–4 |
| | 3 | 6–8 |
| | 3 | over 20 |
| | | *Temperature °F.* |
| | 4 | 100 or less |
| | 5 | 102–103 |
| Fever | 3 | 104–105 |
| | 4 | Present—no record |
| | | *Degree* |
| | 4 | Prominent |
| Exanthem | 7 | Moderate |
| | 5 | Minimal |
| | | *Appearance* |
| Mucous Membrane Lesions (oral) | 3 | Definite |
| | 1 | Doubtful—similar to herpangina |
| Other Features | | Enlargement of tonsillar, cervical or occipital nodes in one-half. |
| | | Chills, headache, myalgia in adults with minimal exanthem. |

* Summary from data published by Neva, Feemster and Gorbach.[10]

Fig. 1.—Epidemic Exanthem. Appearance of exanthem in a 2-year-old patient; fourth day of disease. (Reproduced by permission of J. Am. Med. Assn.)

minimal and occurred regularly after defervescence. The principal manifestations in 16 of the Massachusetts cases are summarized in Table 1. Obviously, the disease in children exhibits certain features similar to rubella, roseola infantum and heat rash and differentially must be distinguished from these conditions.

Feces, throat washings and blood obtained during the acute stage of the illness were inoculated into cultures of various human tissues (Neva and Enders[9]). From 24 of a total of 27 cases thus examined cytopathogenic agents all closely similar in their properties were recovered. In the large majority, these viruses were present in the feces. However, they have also been isolated from the throat of three patients and the blood of one. Isolation from the blood of an agent similar in all respects to those derived from the feces represents, of course, a particularly reassuring part of the evidence for the conclusion that the etiologic agent has been identified.

Introduced into cultures containing human fibroblasts these viruses produce their cytopathogenic effect only after a period of 5 to 7 days. This is a longer *in vitro* incubation period than is characteristic of agents like poliomyelitis virus which may cause cell degeneration within less than 24 hours. In the fibroblastic outgrowth which predominantly occurs in roller tube cultures of human prepucial tissues the cytopathic changes are usually first seen next to the original explant (Fig. 2). Thence they extend toward the periphery of the new outgrowth of spindle cells. The changes consist of rounding, nuclear pyknosis and eventually in disintegration of the entire cell. Epithelial cells which also emerge in these cultures remain normal in appearance as shown in Fig. 3. Similarly in cultures of human renal epithelial cells no cytopathic changes have been observed. The predilection of this virus for fibroblastic elements thus affords a good illustration of the need for using cultures containing various cell types in attempting isolation of unknown viruses.

Virus neutralization tests in tissue cultures were carried out with certain of the agents that were isolated, employing paired sera taken during the acute and convalescent phase of the disease. In this procedure dilutions of the serum to be tested are mixed

TABLE 2.—RESULTS OF SERUM NEUTRALIZATION TESTS ON SERA OF PATIENTS WITH EPIDEMIC EXANTHEM DISEASE FROM WHOSE FECES VIRUS WAS ISOLATED*

| *Patient* | *Strain of Virus Used as Antigen* | *Neutralizing Titers of Patient's Serum* | |
|---|---|---|---|
| | | *Acute Phase Serum* | *Convalescent Phase Serum* |
| M.W. | L.W. | <2† | 32 |
| | V.F. | <2 | 16 |
| L.D. | V.F. | <2 | 16 |
| H.H. | L.W. | <4 | 16 |
| | V.F. | <2 | 8 |
| V.F. | V.F. | <2 | >16 |
| J.C. | L.W. | <2 | >16 |
| | V.F. | <2 | >32 |

* After Neva and Enders.[9]

† Reciprocal of dilution of serum preventing development of cytopathic changes in cultures of human fibroblasts when mixed with 100 $ID_{50}$ of virus. ($ID_{50}$ = amount of virus capable of exerting a cytopathogenic effect in one-half the number of tissue cultures into which it was introduced.)

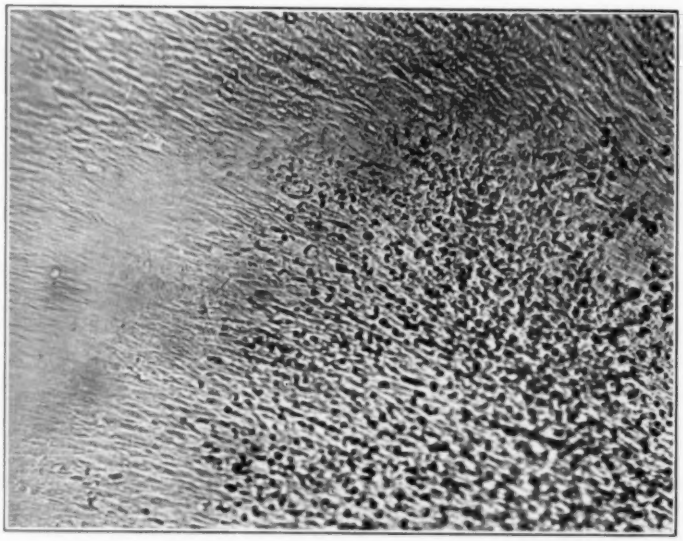

Fig. 2.—Degeneration of fibroblastic outgrowth adjacent to original explant in a roller tube culture of human prepucial tissue following inoculation of virus isolated from patient shown in Fig. 1. Mag. 130 ×. (Reproduced by permission of J. Immunol.)

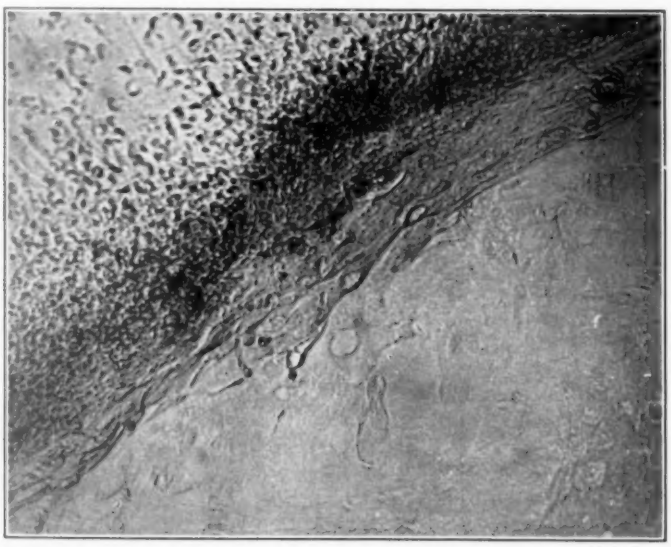

Fig. 3.—Absence of cytopathic changes in sheet of epithelial cell outgrowth (*lower right*) with destruction of fibroblasts (*upper left*) following inoculation of a roller tube tissue culture of human prepucial tissue with virus isolated from patient shown in Fig. 1. Mag. 130 ×. (Reproduced by permission of J. Immunol.)

with a definite number of tissue culture infective doses of the virus. Then each mixture is added to several cultures. If the serum contains antibody specific for the agent under test, cytopathic changes do not appear. In nearly all cases the development of specific neutralizing antibodies was demonstrated in this manner during the course of the illness (Table 2). Cross neutralization tests using the sera of one patient and the virus from another indicated a close similarity or identity of these agents. Using the same technique, Neva[8] has lately found that complete crossing occurs between viruses and sera from the Pittsburgh and Massachusetts epidemics.

The cytopathogenic effects of the agents associated with this disease resemble those produced by certain types of Coxsackie viruses as well as those of the ECHO group. From Coxsackie virus they are distinguished by their lack of virulence for suckling mice. The possibility, however, still remains that they may eventually be classified among certain of the ECHO agents. It would seem, however, that we are justified in drawing the tentative conclusion on the basis of available clinical, epidemiologic and laboratory data that a hitherto unrecognized disease has been delineated which is caused by a viral agent exhibiting a specific antigenic structure and a characteristic cytotropism *in vitro*.

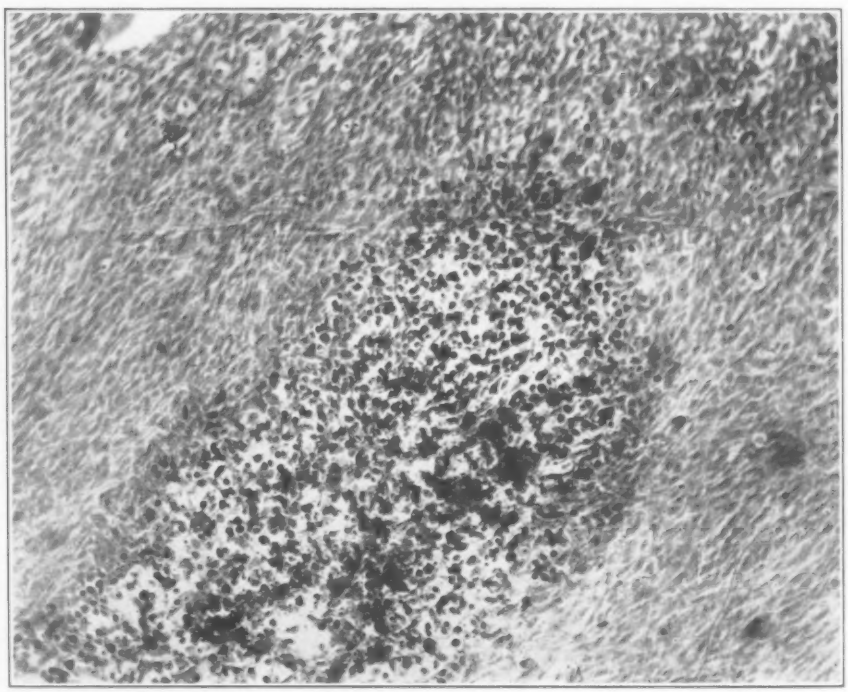

Fig. 4.—Appearance of focal area of cellular degeneration 7 days after inoculation of roller tube culture of human prepucial tissue with varicella agent (H & E Stain). Mag. 100 ×. (Reproduced by permission of Proc. Soc. Exper. Biol. & Med.)

CHICKENPOX. The apparent successful isolation of the varicella virus in tissue culture by Weller[20] in our laboratory

represents the termination of many previous attempts to propagate this agent. Its behavior *in vitro* contrasts sharply with that of the exanthem viruses and, indeed, in one respect which I shall describe immediately, with that of all others we know.

Following the addition of fluid from the vesicles of chickenpox to roller tube cultures of human embryonic skin and muscle tissue or postnatal prepucial tissue, small foci, consisting of abnormal cells, appear scattered at random throughout the layer of normal fibroblastic outgrowth. These cells are easily recognized by increased refractility, rounding of outline and moderate increase in size. As cultivation is continued, the foci enlarge and eventually through confluence, as well as the establishment of new areas of the same sort, all or nearly all the cells become involved and finally disintegrate. Epithelial elements that may be present are likewise attacked by the virus and in general behave in a similar manner. In Fig. 4 is shown, in a stained preparation, a portion of a focus of injured cells adjacent to the layer of normal unaffected cell outgrowth. The extremely sharp boundary between the normal and involved area is noteworthy. Examination under higher power revealed in many cells eosinophilic intranuclear inclusion bodies closely resembling those regularly found in the dermal lesions of the disease itself. The appearance of cells containing such inclusions are shown in Fig. 5.

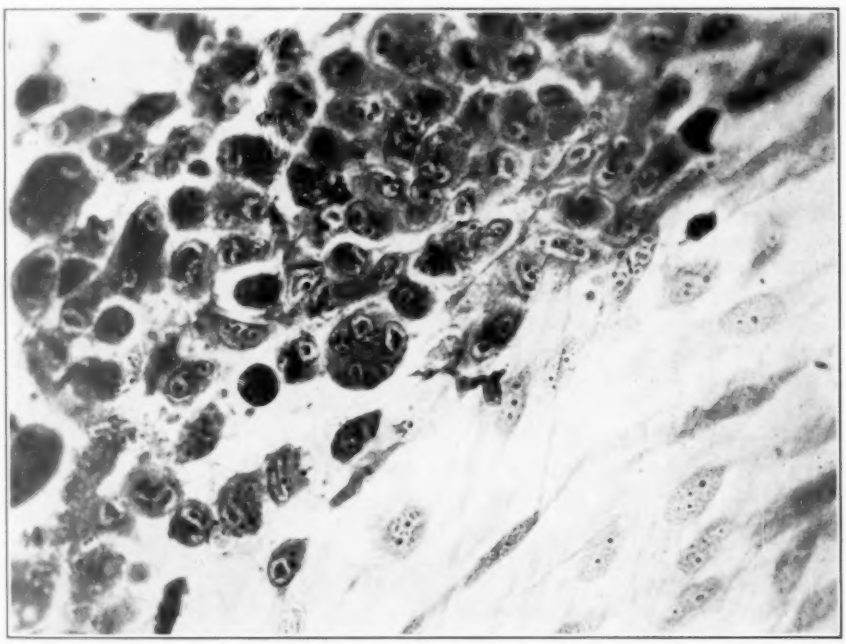

Fig. 5.—Edge of focal area of cellular degeneration. Appearance on sixth day after inoculation of roller tube culture of human embryonic skin-muscle tissue with another strain of varicella agent. Note intranuclear inclusions and multinuclear giant cell. (H & E stain) Mag. 550 ×. (Reproduced by permission of Proc. Soc. Exper. Biol. & Med.)

These very characteristic effects have

been regularly produced with vesicle fluids from a considerable number of typical cases of chickenpox. A few representative strains of the virus have been maintained through many serial passages in tissue cultures over a period of 3 years and have continued to reproduce the same cytopathogenic effects.

A unique phenomenon was revealed when these passages were first inaugurated. It proved essential to include in the inoculum for passage a few infected cells. This finding indicated that the agent, at least in its infective form, was not released into the culture medium as is the case with others which have been studied in tissue culture. Even more surprising was the failure to separate the virus from the cells by a variety of rigorous procedures such as freezing and thawing and grinding with an abrasive. We have here, then, a typical animal virus which, under these conditions, cannot be shown to exhibit infective properties when disassociated from the cell in which it multiplies. The phenomenon of its possible bearing on the problem of viruses as incitants of the malignant state.

In addition to its constant association with the disease and its capacity to reproduce in cell cultures changes characteristic of the dermal lesion *in vivo*, evidence for an etiologic relationship of the agent to varicella has been gained from the results of serologic procedures using acute and convalescent phase sera. Virus neutralization tests have shown the development of an antibody in patients recovering from chickenpox which is capable of reducing significantly the number of foci in tissue culture when mixed with virus (Weller[21]). Application of the Coons' fluorescent antibody technique to cells removed from infected cultures has likewise revealed the emergence of a specific antibody capable of combining with viral antigen within the cell (Weller and Coons[22]). Coons' method consists in applying the serum suspected to contain the antibody to fixed and dried tissue cells containing the antigen. Excess serum is removed by washing. The preparation is then treated with a fluorescent antibody solution specifically reacting with the gamma globulin of the serum under examination, in this case human gamma globulin. The complex of antigen and antibody is rendered in this manner visible under the fluorescent microscope. In the discussion of our work on measles a figure illustrating this effect is included (Fig. 9).

From the lesions of cases of herpes zoster, Weller[20] has in the same manner isolated a number of agents which produce cytopathic changes indistinguishable from those caused by the varicella virus as shown in Fig. 6. Acute and convalescent phase sera from cases of varicella and herpes zoster and those obtained at various times from cases of recurrent herpes simplex were applied to tissue cells infected with the respective viruses in a variety of combinations as shown in Table 3 and examined by means of the Coons' technique. It is clear that the varicella and zoster sera exhibited marked cross reaction with sera taken during the convalescent phase of each disease while the sera from patients with herpes simplex failed to react with the viruses of zoster or varicella. Results of complement fixation tests[21] using antigen prepared in tissue cultures, which I shall not present in detail, have likewise afforded additional evidence for the close antigenic relationship of identity between these two viruses. Experiments are also in progress in which the capacity of convalescent sera to cross neutralize the cytopathogenic effects of the respective agents is being tested. If the expected results are obtained

TABLE 3.—RESULTS OF EXPOSING TISSUE CULTURE CELLS INFECTED WITH THE VIRUSES OF VARICELLA, HERPES ZOSTER, AND HERPES SIMPLEX TO SERA FROM PATIENTS WITH THESE DISEASES AND SUBSEQUENTLY TO FLUORESCENT ANTI-HUMAN GAMMA GLOBULIN RABBIT SERUM (COONS' TECHNIQUE)*

| | | *Results in Cells Infected with Virus of* | | | | | |
|---|---|---|---|---|---|---|---|
| | | *Varicella* | | | *Herpes Zoster* | | *Herpes Simplex* |
| *Source of Serum* | *Day of Illness Serum Obtained* | *McE.*† | *Wel.* | *Pat.* | *Blu.* | *Sto.* | *Rod.* |
| *Varicella* | | | | | | | |
| Case 1 | 3 | 0 | 0 | ± | + | 0 | ± |
| | 9 | 4+ | 4+ | 4+ | 4+ | 4+ | + |
| Case 2 | 1 | ± | 0 | 0 | 0 | 0 | + |
| | 7 | 4+ | 4+ | 4+ | 4+ | 4+ | + |
| *Herpes Zoster* | | | | | | | |
| Case 1 | 2 | 0 | ND | ND | 0 | 0 | ± |
| | 51 | 3+ | ND | ND | 2+ | + | ± |
| Case 2 | 4 | ± | ND | ND | 0 | 0 | 4+ |
| | 27 | 3+ | ND | ND | 4+ | 4+ | 4+ |
| *Herpes Simplex* | ‡ | | | | | | |
| Case 1 | A | ND | ND | ± | ND | ND | 4+ |
| | B | ND | ND | ± | ND | ND | 4+ |
| Case 2 | A | 0 | ND | ND | 0 | 0 | 2+ |
| | B | ± | ND | ND | 0 | 0 | 3+ |

* Data from Weller and Coons.[22]
† Denotes strain of virus employed.
‡ Sera from cases of recurrent herpes simplex taken at varying intervals not related to appearance of typical lesions.

Fig. 6.—Intranuclear inclusions in cell outgrowth of human embryonic skin-muscle in roller tube culture 2 days after inoculation with herpes zoster agent. (H & E stain) Mag. 825 ×. (Reproduced by permission of Proc. Soc. Exper. Biol. & Med.)

it may be affirmed with much confidence that these two viruses are one and the same. Absolute proof, of course, can be obtained only by inoculation of man. Such experiments, however, are not contemplated at the present time.

MEASLES. Over 15 years ago my associates at that time and I spent months in attempting to find ways of propagating the virus of measles in monkeys, chick embryos, chick embryo tissue cultures and even in cultures consisting of human placental tissue which unfortunately did not grow well. On the whole we were unsuccessful, although we did, in accordance with the observations of previous workers, note a scanty rash in an occasional monkey following inoculation with blood or throat washings from patients with the disease. After we had shown that poliomyelitis could be cultivated in a variety of human tissues the urge to try the same system with measles virus became irresistible, since in spite of intervening reports of its cultivation, none had been generally confirmed. Accordingly, with Peebles, we introduced into roller tube cultures of human kidney tissue blood or throat washings taken from typical cases during the first 24 hours of the rash. After a week or longer we found to our delight changes of a peculiar sort[11]. As seen under low power these consisted of circumscribed areas in the cell outgrowth containing nuclei and cytoplasm but in which the cell boundaries had entirely disappeared. Upon continued incubation the cytoplasmic portion became increasingly vacuolated until it presented a torn or foamy appearance. In fixed and stained preparations these areas were seen to represent syncytia or large giant cells which contained 40 or more nuclei. Within most of the nuclei typical eosinophilic inclusion bodies were present. The process proceeds in a leisurely manner to involve ultimately the whole layer of newly grown cells. These interesting cytopathic changes are illustrated in Figs. 7 and 8. They are, however, not exclusively characteristic of the measles agent, for it has been subsequently shown by Rustigian[16] that viruses latent in the kidney of rhesus monkeys may multiply in tissue cultures and produce very similar effects when these are observed in the fresh state. So far, however, in our experience inclusion bodies in the nuclei of the syncytial formations have not been observed after inoculation of tissue cultures with Rustigian's monkey viruses. In stained preparations, therefore, they have been easily differentiated from the measles agent.

As I have just implied, we believe that the virus which produces the effects in human renal cells we have described represents the etiologic agent of measles. The evidence for this belief I shall, in conclusion, very rapidly summarize.

Strains exhibiting identical properties have been isolated from the blood of 8 patients with typical measles. Some of these were isolated in 1954 and others in 1955. One other strain was obtained from the lung tissue of a fatal case.

Viral neutralizing antibodies capable of preventing cytopathic changes have been shown to develop regularly and promptly in nearly all cases of measles that we have examined. Examples of the titers obtained are given in Table 4. In the last instance it will be noted, the titer had probably reached its maximum at the time the first blood was withdrawn.

An antigen appears in tissue cultures which fixes complement with convalescent sera, but either not at all with acute phase sera or, if so, only to a lower titer. The results of complement fixation tests on the paired sera of 25 cases are summarized in Table 5. It

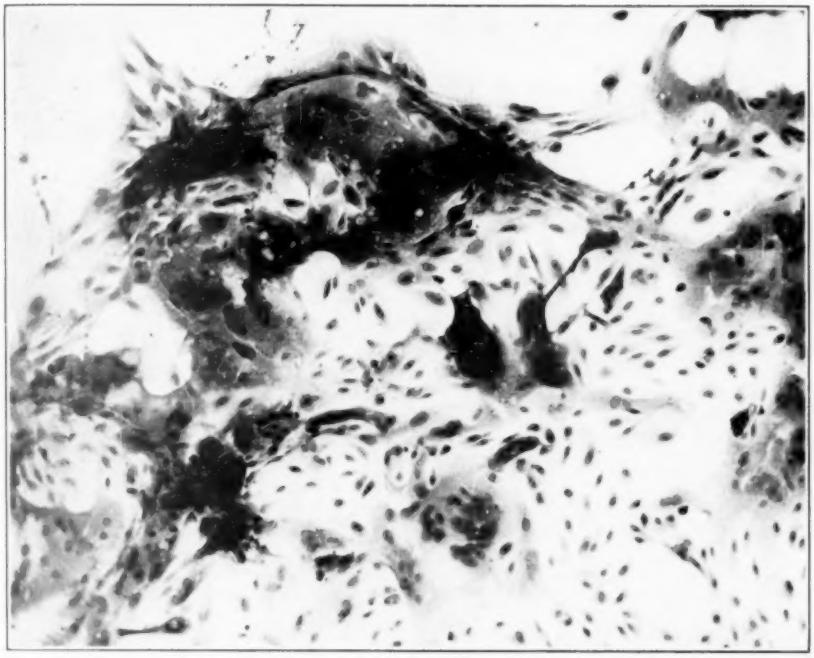

Fig. 7.—Appearance of cytopathic changes in human renal cells 20 days following introduction of measles virus into a roller tube culture. Note syncytial or "giant cell" formation. (Fixed in formalin. H & E stain). Mag. approx. 100 ×.

Fig. 8.—Syncytium or "giant cell" in outgrowth from human kidney tissue in a roller tube culture 18 days after inoculation of measles virus from the fourth serial tissue culture passage. (Carnoy's fixative. H & E stain). Mag. approx. 800 ×.

is evident that an increase of four times or more, which is regarded as significant, was recorded in 22 instances.

In the sera of persons giving a positive history of measles this complement fixing antibody has usually been found whereas in those with a negative history, it has seldom been present. In Table 6 the findings in 30 individuals are recorded. Additional data not included in the table are in general confirmatory. A few deviations, however, have been observed from the expected correlation. In view of the difficulty of the accurate diagnosis of measles in all instances, such discrepancies, perhaps, are not surprising.

Antibody reacting with the virus has also been shown to appear in convalescent phase sera which can be demonstrated by means of the Coons' technique[18]. The appearance of the characteristic giant cells after application of the serum and subsequent treatment with fluorescent anti-globulin anti-

TABLE 4.—RESULTS OF VIRUS-NEUTRALIZATION TESTS IN CULTURES OF HUMAN KIDNEY TISSUE ON SERA FROM PATIENTS WITH MEASLES

| | *Serum* | |
|---|---|---|
| *Patient* | *Acute Phase** | *Convalescent Phase†* |
| I | <4‡ | 128 |
| II | <4 | 128 |
| III | =>64 | 128 |

* Acute phase sera obtained from about 24 to 96 hours after appearance of exanthem.
† Convalescent sera obtained about 14 days after appearance of exanthem.
‡ Reciprocal of serum dilution preventing typical cytopathic changes when mixed with 100 $ID_{50}$ tissue culture doses of virus.

TABLE 5.—SUMMARY OF RESULTS OF COMPLEMENT FIXATION TESTS ON ACUTE AND CONVALESCENT PHASE SERA OF PATIENTS WITH MEASLES

| | *Number in which CF Antibody Increased During Period Studied** | |
|---|---|---|
| *Total Cases* | *4× or more* | *2× at least* |
| 25 | 22† | 3 |

* CF Tests carried out according to method of Fulton and Dumbell as modified by Svedmyr, Enders and Holloway.[18] Undiluted nutrient fluid removed from tissue cultures infected with measles virus was employed as antigen.
† An increase of 4× is considered significant. The mean increase in these patients was 31×.

TABLE 6.—SUMMARY OF RESULTS OF COMPLEMENT FIXATION TESTS ON SERA OF PERSONS WITH POSITIVE OR NEGATIVE HISTORIES OF MEASLES

| | | *Number Yielding Sera with CF Antibody Titer of* | |
|---|---|---|---|
| *History of Measles* | *Number Persons Studied* | *4 or lower** | *8 or higher* |
| Positive | 17 | 3 | 14 |
| Negative | 13 | 13 | 0 |

* Reciprocal of serum dilution fixing 2 units of complement in the presence of nutrient medium from cultures of human kidney cells infected with measles virus.

body* is illustrated in Fig. 9. Acute phase sera in general do not produce this effect. The prominence of the fluorescent antigen-antibody complex in the cytoplasm was unexpected in view of the fact that the intranuclear inclusion body represents the striking feature in cells stained by the ordinary method.**

More indirectly, the finding of high titers of both neutralizing and complement fixing antibodies which we have found in specimens of human gamma globulin supports the thesis that the agent is the cause of measles. In view of the marked prophylactic effect of this material, high concentrations of antibody for the virus of measles would be expected.

Finally in Cynomolgus monkeys a disease which in all essential respects closely resembles measles in man has followed inoculation of virus from the 23rd serial tissue culture passage of the virus. In Fig. 10, the data obtained in one animal are presented as an example. The virus was introduced by both the intranasal and intravenous

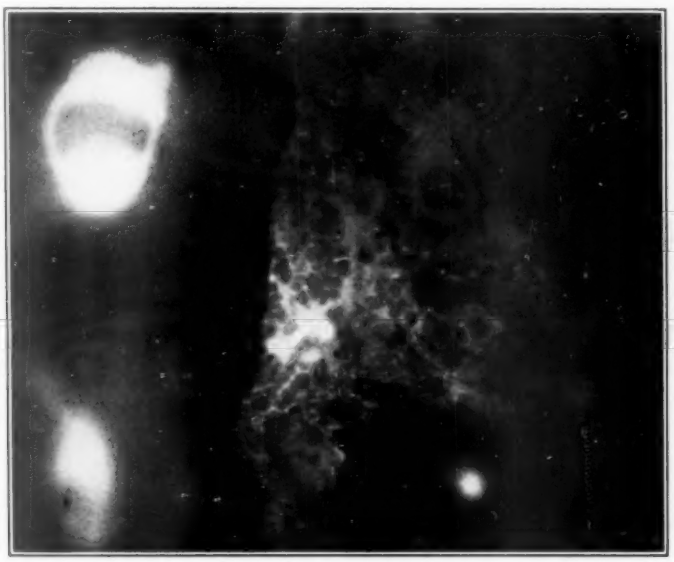

Fig. 9.—Appearance of syncytium from a roller tube culture of human kidney tissue 7 days after inoculated with measles virus (Edmonston strain 12th tissue culture passage), treated with convalescent phase measles serum followed by fluorescent anti-human gamma globulin rabbit serum. Note that the fluorescent material is largely distributed between the nuclei. The large very bright oval body is a bubble. Mag. 100 ×.

*The experiments employing the fluorescent antibody technique were carried out in collaboration with Dr. Albert Coons and Dr. Barbara Watson of the Department of Bacteriology and Immunology of the Harvard Medical School and Dr. Gisela Ruckle now of the Department of Preventive Medicine, University of Pittsburgh Medical School.

**Cohen and her collaborators[1] have recently isolated viruses from cases of measles which conform in general in their properties with those of our agents. Antibody developing in human convalescent measles sera was demonstrated by the Coons' technique using cultures of infected monkey renal cells. In certain instances, considerable quantities of fluorescent material were revealed within the nucleus.

routes. Thereafter, no virus was recovered from the blood until the fifth day when it appeared and persisted until the 14th day. From the 11th to the 15th day a rash was observed on the face, trunk and extremities which closely resembled the exanthem seen in man. Within a day or two after the disappearance of the rash and within 4 days after the cessation of viremia, complement fixing antibody appeared in the blood which reached maximum

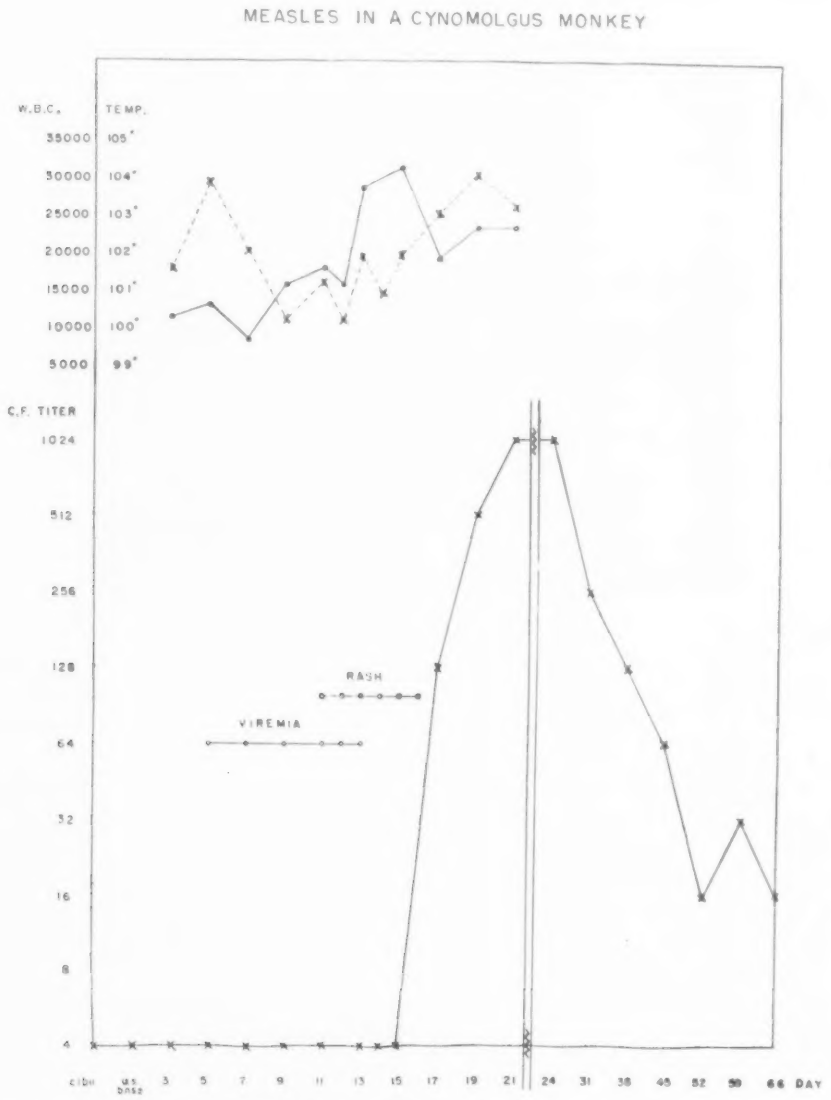


Fig. 10.—Data obtained following inoculation of a Cynomolgous monkey with measles virus (Edmonston strain 23rd passage in human kidney tissue culture). Undiluted tissue culture 0.75 ml. given intravenously and 0.25 ml. intranasally. WBC x---x; temp. •——•; titer comp. fix. antibody x——x.

titer within a few days. The concentration of this antibody declined rather rapidly during the ensuing weeks.

These phenomena have not been seen in every monkey injected with the virus. In some the rash has been absent or minimal, although viremia has occurred. In one animal neither rash nor viremia was noted. In every case, however, antibody has developed. So far we have failed to cultivate the virus in chick embryos or chick embryonic tissue cultures. We are inclined, therefore, to believe that this agent does not multiply under these conditions as reported by previous investigators. In the renal cells of monkeys, however, multiplication has been shown to take place accompanied by cytopathic changes.

In conclusion I would point out that if we are correct in thinking we are in possession of the etiologic agent of measles we have now the means of directly assaying the protective antibody in human globulin. Furthermore, the possession of the virus in a form that can be manipulated in the laboratory may lead eventually to the development of a practical way of inducing active immunity.

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*(Interlingua on page 658)*

# CLINICAL MANIFESTATIONS OF OZONE POISONING: REPORT OF A NEW SOURCE OF EXPOSURE

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The potential hazard for human injury arising from exposure to ozone is receiving greater recognition. This is reflected by a growing literature describing the increasing sources of human exposure to ozone, its technical control and the physiologic and toxicologic effects following acute and chronic exposure both experimentally and in man[2,4,6,7,8].

The most pronounced deleterious effect of ozone inhalation is on the respiratory system. As observed experimentally this consists of pulmonary edema and hemorrhage, resulting in death if the exposure is sufficiently severe, or temporary pulmonary embarrassment with recovery if less severe. No death from exposure to ozone in man has been reported. Secondary effects consist of the irritation of the mucous membranes, headache and lethargy. Low concentrations have a narcotizing and general depressant effect, producing sleep in certain individuals similar to what has been observed in animals[3,5].

Physiological studies have demonstrated that ozone causes a decreased rate of pulmonary ventilation and lowered output of carbon dioxide, but the diminution in carbon dioxide is less than that of oxygen, resulting in a respiratory quotient which may be greater than one[1].

The purpose of this paper is twofold: 1) to describe 3 cases of ozone toxicity following subacute exposure and the problems of differential diagnosis presented and 2) to report a new source of human exposure to ozone.

**Case Reports.** CASE 1. L.P., a 51-year-old male, employed as a welder, was seized suddenly while at work by severe headache, dyspnea and substernal oppression. These symptoms shortly increased in severity and the patient had a sense of impending doom. A physician, summoned to see the patient, suspected an acute myocardial infarction and had him hospitalized.

The patient had an attack of "influenza" in 1953. His general health otherwise had been good.

*Occupational History.* He has been a welder for the past 10 years. Recently a new technique of electric welding known as *consumable electrode gas-shielded arc welding* was instituted at his place of employment and he had been utilizing this for 2 weeks prior to his present complaints. This welding process uses a continuously fed bare wire as one of the electrodes which is of the same composition as the material welded. The electrode is melted and consumed in the process and requires current up to 500 amperes.

On admission, the patient was observed to be a well developed and well nourished cyanotic male, in acute respiratory distress.

Clinically, the neck veins were not congested. Adventitious sounds were heard over both lungs, but rales were absent. The heart was not enlarged, the heart sounds of good quality and no murmurs were heard. Examination of the abdomen and extremities revealed no abnormality. The reflexes were normal. The temperature was 98.2° F., pulse 115 per minute and regular, and blood pressure 130/80. He was placed in an oxygen tent and given morphine, aminophylline and penicillin.

*Laboratory Data.* Chest roentgenogram taken on admission showed increased bronchovesicular markings; small densities throughout both lung fields and an area of consolidation in the left lower lobe (Figs. 1*A* and 1*B*). Other laboratory data, including electrocardiogram, serology, sedimentation rate, red and white blood count, were all normal. Urine analysis showed a proteinuria (3+) and scattered white cells, red cells and finely granular casts.

*Course.* The day following admission, the patient was severely dyspneic and cyanotic when out of the oxygen tent. Clinically, moist rales were present throughout both lung fields. He showed little improvement in the next 2 days during which he developed a moderately severe hacking cough. Sputum culture yielded *Candida albicans* but was negative for tubercle bacilli. After the fifth hospital day, he began to improve, with gradual subsidence of symptoms and disappearance of rales in his chest. The chest roentgenogram taken two days prior to discharge showed complete clearing of the lung fields. A repeat white blood count and differential count were normal, and urine test for albumin and microscopic examination of sediment were negative. He was discharged on the 13th hospital day and he returned to work after one month. Nine months later (July 8, 1955) he was still complaining of moderate fatigue, exertional dyspnea and a mild nonproductive cough. With the exception of notable weight loss, the clinical examination did not disclose any significant abnormalities.

CASE 2. J.V., a 42-year-old white male, employed as a welder, experienced burning of his eyes and throat followed by a nonproductive cough associated with an intermittent choking sensation. These symptoms persisted and several days later he complained of dizziness, an acrid taste and shortness of breath. The latter became severe, and he was hospitalized.

With the exception of a previous hospitalization for pneumonia and an allergy to various foods, the patient had been in good health.

*Occupational History.* For the past 20 years the patient has been employed as a grinder and as an electric welder, the latter only recently. The operation of electric welding was of the same type as employed by L.P. (Case 1), and he had used it for 3 days prior to his present illness.

On admission, the patient appeared thin and emaciated, and was coughing a great deal. On clinical examination the chest disclosed bronchial breathing and scattered coarse rales over both lung fields. The heart was not enlarged; the sounds were of good quality and no murmurs were audible. The abdomen and extremities were normal. There were no abnormal neurological signs.

*Laboratory Findings.* Peripheral blood examination showed a hemoglobin of 101%; red blood cells, 4.9 million/c.mm. total white count 8200/c.mm. with the following differential: stabs, 6%; polymorphonuclear leukocytes, 71%; lymphocytes, 16%; monocytes, 3%; eosinophils, 4%. Urine analysis for sugar and albumin was negative and the sediment showed scattered white cells and an occasional red cell per high power field. A chest roentgenogram showed small areas of increased density throughout both lung fields and increased bronchial vesicular markings. The heart was of normal size and contour.

*Course.* The patient was placed on achromycin and supportive therapy. He showed steady improvement with subsidence of cough, clearance of abnormal chest signs and disappearance of small densities on follow-up roentgenogram. He was discharged on the ninth hospital day, still complaining of some weakness. He returned to work after 7 weeks and has been continually employed since. Re-examination 9 months later (July 8, 1955) did not disclose any abnormalities.

CASE 3. W.W., a 31-year-old white male welder, complained of an acrid taste and smell associated with anorexia after leaving work. Shortly thereafter, he had a shaking chill and fever (102° F.). This was followed by a severe pounding occipital headache, dyspnea and moderate substernal pressure. The next day he felt somewhat better but consulted his physician who diagnosed his illness as an upper respiratory infection and administered penicillin.

With the exception of influenza in 1952 and a left herniorrhaphy at age seven, the patient had had no serious illnesses. Systemic review was not contributory.

*Occupational History.* The patient had been an electric welder for the past 9 years. Prior

A

B

Fig. 1*A*.—Posteroanterior film of the chest taken on admission in Case 1, showing increased bronchovesicular markings. Small densities in both lungs and an area of consolidation in the left lower lobe.

Fig. 1*B*.—Left lateral view of the chest in Case 1, showing area of consolidation in posterior segment of lower lobe.

to that he was in military service for 2 years. This patient likewise changed over to the new welding technique as described above, and had been utilizing it for 2 weeks.

*Course.* The patient improved, although his cough persisted. Three days after the onset of the above symptoms he felt well enough to return to work. Re-examination revealed that the patient still had some wheezing on the right side of chest, but no rales or rhonchi. A chest roentgenogram showed increased hilar shadows bilaterally and faint mottled densities in both lung fields, more on the right. Peripheral blood examination disclosed a hemoglobin of 14 gm., a red blood count of 5.1 million, and a white blood count of 8300 with a normal differential. Urine analysis for sugar and albumin and examination of sediment were negative. Re-examination 9 days later disclosed that the lungs were entirely clear on auscultation as well as on roentgenogram. A follow-up 9 months later (July 10, 1955) showed the patient to be in excellent health.

**Discussion.** It is noteworthy that although the clinical pictures presented by the 3 patients were similar, none of the presenting symptoms or clinical findings were diagnostic. The difficulty in establishing a diagnosis may be appreciated in view of the number of clinical entities which resemble ozone toxicity, prominent among which are: a) pulmonary inflammatory disease due to inhalation of other toxins, such as nitrous fumes and phosgene; b) acute myocardial infarction; c) pulmonary infarction; d) congestive heart failure; e) bronchial asthma and f) lobular or bronchopneumonia.

In essence, the diagnosis of ozone toxicity requires a high index of suspicion, an adequate occupational history, a knowledge of environmental exposure, familiarity with toxicological effects and the finding of significant amounts of ozone in the breathing zone of the worker. It is noteworthy that in the cases described above the diagnosis was not suspected until after the third case appeared within a relatively short period, including only welders and all from the same plant.

Other intoxications such as nitrous fumes and phosgene were ruled out after analyses of the air at the breathing zone of these workers during the performance of this specialized welding technique known as consumable electrode gas-shielded arc welding. The average concentration of ozone found was excessive (9.2 ppm in working environment*), with only a trace of nitrous fumes and no phosgene. Acute myocardial infarction, strongly suspected on admission in Case 1 (L.P.), was excluded in the light of the subsequent clinical course and a normal electrocardiogram. With the exception of a lobular or bronchopneumonia, the other clinical entities enumerated above appear untenable in any of the cases in view of the environmental history, the clinical course and the discovery of high concentrations of ozone. The chest roentgenograms in J.V. and W.W. were consistent with either a bronchopneumonia, pulmonary edema or pneumoconiosis. The clearing of the roentgenographic shadows excluded the latter. The negative cardiac history and normal electrocardiogram likewise ruled out pulmonary edema. The well known fact that the pulmonary inflammatory disease produced by ozone may be complicated by a bronchopneumonia or lobular pneumonia made their chest roentgenograms compatible with the diagnosis of ozone intoxication.

A number of interesting and significant aspects presented by these patients are worth mentioning. In terms of severity, it should be noted that both L.P. and J.V. showed what are probably the most severe clinical courses yet reported. This may well be due to the high content of ozone in the breathing area of the workers for a prolonged

*The present threshold limit according to most recent acceptable standards for ozone is 0.1 part per million in the working environment.

period and in the absence of protective measures. Another feature is the disproportion between the severity of the initial symptoms, such as dyspnea and substernal oppression, and the minimal clinical findings in the chest. Two factors may account for this: 1) the respiratory distress may be partly due to the direct stimulation of the pulmonary receptors and 2) the absence of rales as an early finding in L.P. may be explained on the basis of a bronchospasm.

It is difficult to explain the transient renal injury manifested by proteinuria and the abnormal microscopic elements found in L.P., especially since no toxic substance has been found in the circulating blood following repeated experimental exposure to ozone[3]. A striking feature presented by all 3 patients is the rather slow recovery following removal from exposure. Although it is well recognized that ozone exposure is usually associated with a delayed onset of symptoms requiring a period of enforced inactivity, the morbidity attached to the disease has not been sufficiently stressed in the literature. The residual symptoms of fatigability, weakness, exertional dyspnea and weight loss presented by two of these patients illustrate this important point.

In view of the lack of any specific treatment of ozone toxicity, the most effective measure is that of prevention and this requires engineering control. The latter includes isolation of the process, preferably in special booths, and the provision of local exhaust ventilation at the source. When significant exposure is suspected, precautionary measures such as reduction of physical activity or bed rest are advisable. The treatment of a specific case is essentially symptomatic and includes such measures as 1) bed rest, 2) oxygen, 3) antipertussants, 4) analgesic for the headache and chest pain and 5) antibiotics. In view of the respiratory depression in certain patients morphine is ill advised.

**Summary and Conclusions.** Three cases of ozone poisoning occurring in welders employing a new welding technique known as consumable electrode welding are reported.

The difficulty in diagnosis is apparent in view of the number of clinical conditions which resemble ozone poisoning, namely: a) acute pulmonary edema, b) pulmonary infarction, c) acute myocardial infarction, d) bronchial asthma, e) bronchopneumonia and f) pulmonary inflammatory disease due to other toxins, such as nitrous fumes and phosgene.

The diagnosis of ozone poisoning requires a high index of suspicion, an adequate occupational history, familiarity with the toxicological effects and finding significant amounts of ozone in the working environment. The presence of 9.2 parts per million (ppm) of ozone at the work site was far in excess of the presently accepted threshold limit of 0.1 ppm.

The following interesting features are stressed: a) severity: in 2 patients the clinical courses were the most severe reported; b) disparity between symptomatology and clinical findings: the marked dyspnea, chest pain and cough were accompanied by only minimal findings in the chest; c) morbidity: the prolonged morbidity after complete clearance on chest roentgenogram was notable in two. This feature has not been stressed to date.

Since treatment is symptomatic, the main emphasis is on prevention and requires engineering control. The latter includes isolation of the process and provision of local exhaust ventilation.

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## SUMMARIO IN INTERLINGUA

### Manifestationes Clinic de Invenenamento a Ozono: Reporto de un Nove Situation de Contacto

Es presentate tres casos de invenenamento a ozono occurrente in obreros qui empleava un nove technica de soldatura cognoscite como "soldar a consumption de electrodo."

Le difficultates diagnostic es apparente si on considera le multiplicitate del conditiones clinic que es simile a invenenamento a ozono. Illos include (a) acute edema pulmonar, (b) infarcimento pulmonar, (c) acute infarcimento myocardial, (d) asthma bronchial, (e) bronchopneumonia, e (f) morbo inflammatori pulmonar debite a altere toxinas como per exemplo vapores nitrose e phosgeno.

Le diagnose de invenenamento a ozono presuppone un alte indice de suspicion, un compatibile historia occupational, familiaritate con le effectos toxicologic, e le demonstration de significative quantitates de ozono in le ambiente occupational. Le presentia de 9,2 pro million de ozono, que esseva constatate per nos al sito de labor, excede grandemente le currentemente acceptate limine maximal de 0,1 pro million.

Le sequente aspectos es de interesse special. (1) Severitate: In duo de nostre patientes le curso clinic esseva le plus sever unquam reportate. (2) Disparitate de symptomatologia e constatationes clinic: Le marcate dyspnea, dolor thoracic, e tusse esseva accompaniate de solmente minime constatationes in le thorace. (3) Morbiditate: In duo del casos il esseva notabile que le morbiditate se prolongava post le complete clarification del roentgenogramma thoracic. Iste aspecto ha non previemente essite signalate.

Proque le tractamento es symptomatic, le investigation del problema debe concentrar se plus tosto super mesuras preventive. Istos es de natura technic e include le isolation del processo de soldatura e le provision de ventilation local.

# THE EFFECT OF CORTICOTROPHIN (ACTH) ON AMMONIA PRODUCTION IN THE NEPHROTIC SYNDROME*

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The nephrotic syndrome includes numerous physiologic defects. Corticotrophin (ACTH) and adrenal corticosteroids have favorably influenced some of these defects. The mechanism by which these hormones produce their beneficial results is not understood. Recent writings have focused attention upon the direct action of these hormones on renal cellular physiology.

The purpose of this paper is to report the significant rise in ammonia ion excretion which occurred in a series of patients with the nephrotic syndrome who responded well to ACTH administration. It is most probably another demonstration of the effect of these hormones on renal tubular enzyme systems.

**Methods and Materials.** Ten patients with the nephrotic syndrome receiving ACTH and in whom adequate urine collections could be made were studied. They ranged in age from 4 to 28 years. The diagnosis of the nephrotic syndrome was based on conventional criteria. Eight of these patients responded well to ACTH. Two (Figs. 8 and 9) failed to respond and died in uremia shortly after this recorded period. All but one of the 8 successes have been well for a period varying from 3 months to 3 years. They have been maintained on a low-sodium, acid-ash, high-fluid regimen[22,23,24,25,26]. The one patient in this group who did not do well left our care and was treated by a nonmedical practitioner and died.

Metabolic studies were carried out on a metabolic unit with its own diet kitchen and laboratory. The reader is referred to a previous publication for details as to its organization and as to studies routinely undertaken in edematous subjects[26]. This report is primarily concerned with the level of ammonia excreted in the urine. Urine ammonia determinations on daily 24-hour urine samples were done according to the method of Folin as modified by Van Slyke and Cullen[27]. Daily balance studies for sodium and potassium were undertaken. The daily dietary intake of sodium and potassium was calculated from data supplied by Bowes and Church[3] for the food actually ingested. Urine and plasma sodium was determined by the use of the Beckman flame photometer. Fecal and skin losses were estimated according to a report published by Arn and Reimer[1]. Body weights were recorded on a scale sensitive to 100 gm.

**Case Reports.** CASE 1. Patient B.E. (Fig. 1) is a 5-year-old male who developed edema in the summer of 1951. His initial course was variable in intensity, but edema and ascites became progressively more severe. He was first seen by us in December, 1953, at which time he was markedly edematous, had ascites, and showed a marked anemia. He was hospitalized for a period of 98 days and his course was marked by many exacerbations associated with repeated upper respiratory infections. A 7-day course of ACTH in February, 1954, was successful. He was again admitted in July, 1954, after he had developed a slowly progressive edema and diarrhea following an upper respiratory infection. It is of interest to note that the diarrhea which responded poorly to the usual measures stopped after 24 hours of ACTH administration.

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CASE 2. Patient R.E. (Fig. 2) is an 11-year-old female whose edema was first noted in January, 1953. Her course was progressive and she received ACTH as diagramed in April of 1953. Since that time she has remained stable on a high-fluid, acid-ash, low-sodium regimen.

CASE 3. Patient C.D. (Fig. 3) is a 4-year-old male in whom symptoms of edema began in September, 1951. His course was severely progressive and he received ACTH therapy in November, 1951, with a gratifying response. He was lost from observation shortly thereafter and died under treatment by a nonmedical practitioner.

CASE 4. Patient M.O'L. (Fig. 4) is a 9-year-old female who was admitted in August, 1953. She had a history of edema beginning in November, 1950. She was fairly well controlled by an acid-ash, high-fluid regimen, but developed repeated episodes of upper respiratory infection and increasing edema

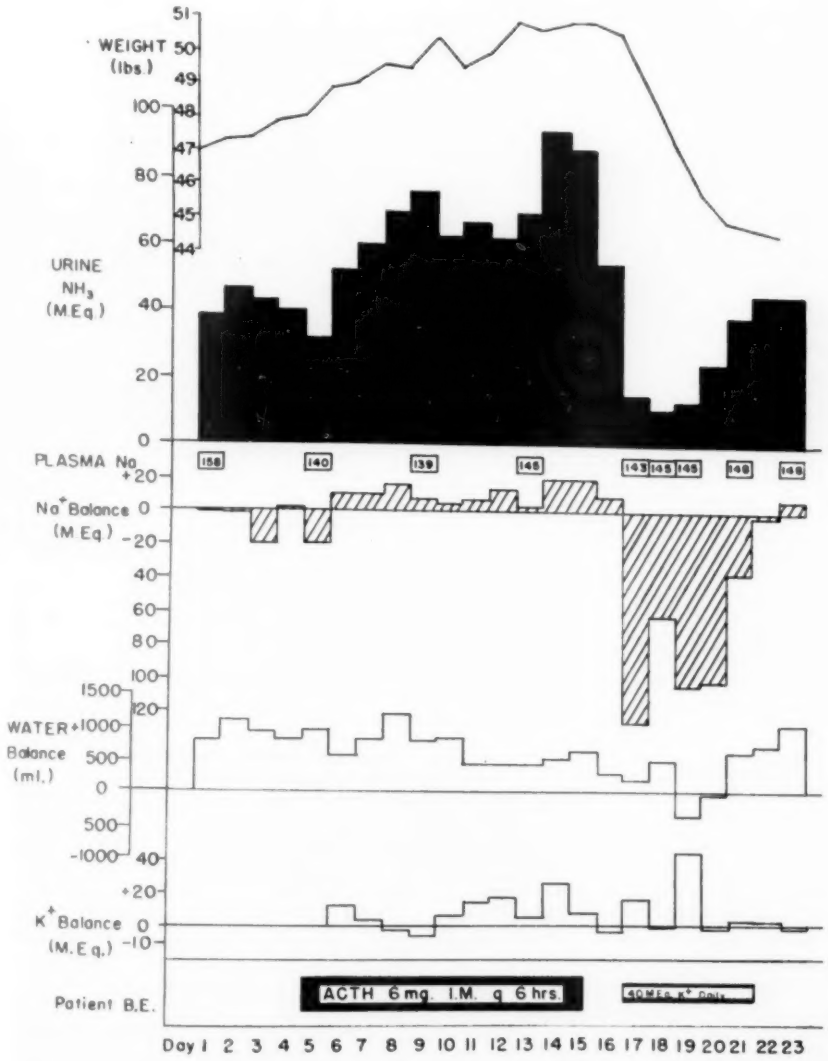
WEIGHT (lbs.)
URINE NH3 (M.Eq.)
PLASMA Na
Na+ Balance (M.Eq.)
WATER Balance (ml.)
K+ Balance (M.Eq.)
Patient B.E.
ACTH 6 mg. I.M. q 6 hrs.
Day 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23

Fig. 1.

early in 1953. A course of ACTH was given in August, 1953, with good results and the patient has been well and active since that time.

CASE 5. Patient J.M. (Fig. 5) is a 28-year-old female who first became edematous in November, 1949. She received a course of ACTH in August, 1951, with a loss of edema and a general improvement in clinical condition. She has been maintained edema-free and with a stable clinical course since that time.

CASE 6. Patient L.A. (Fig. 6) is a 7-year-old male first seen in the summer of 1948 with a beginning nephrotic syndrome. He was maintained relatively edema-free with the exception of occasional periods of exacerbation until November, 1951. An upper respiratory infection initiated a marked generalized edema. He responded very well to ACTH. Since then he has been well by all clinical standards and maintains an almost normal existence.

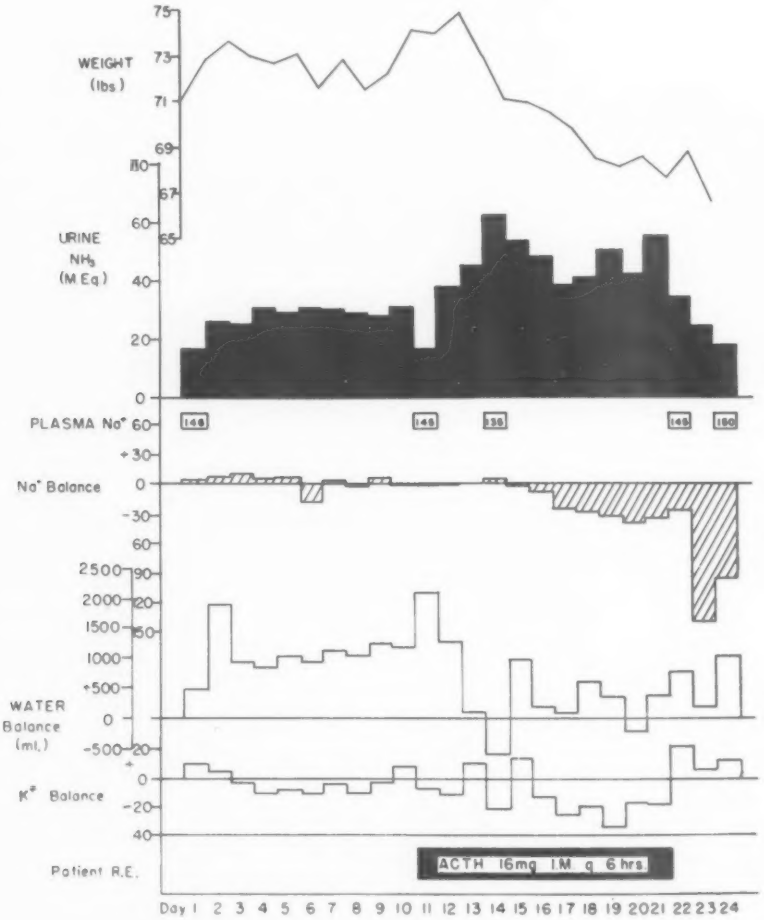


Fig. 2.

CASE 7. Patient V.M. (Fig. 7) is a 5-year-old female whose edema was first noted in October, 1949. In October, 1951, a severe upper respiratory infection initiated severe generalized edema. She responded well to ACTH. She has been maintained relatively stable since that time.

CASE 8. Patient L.D. (Table 2) is a 19-year-

old male admitted to the unit in October, 1954, with a history of steadily progressive edema for a period of 6 months. He had received a course of cortisone therapy in relatively large doses for a 2-week period about 2 months before his admission. His clinical state failed to improve during cortisone therapy. After an 8-day control period, ACTH therapy was begun due to his poor physical condition. Loss of 43 pounds of edema fluid followed ACTH therapy.

CASE 9. Patient P.T. (Fig. 8) is a 14-year-old male who showed evidences of uremia at the age of 3½. He had a long history of repeated episodes of pyelonephritis and edema. He was first seen at the Metabolic Unit in November, 1948. Renal function studies at that time showed markedly decompensated

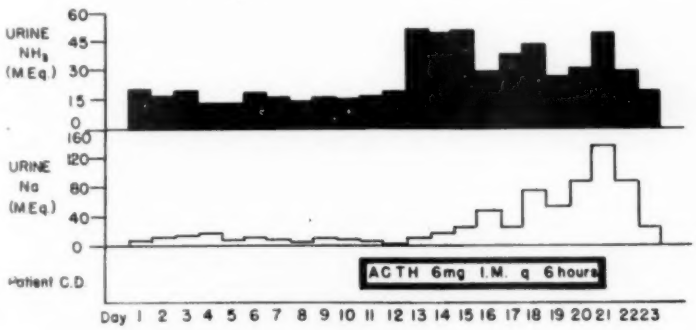


Fig. 3.

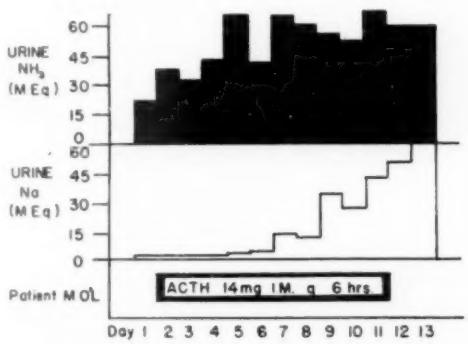


Fig. 4.

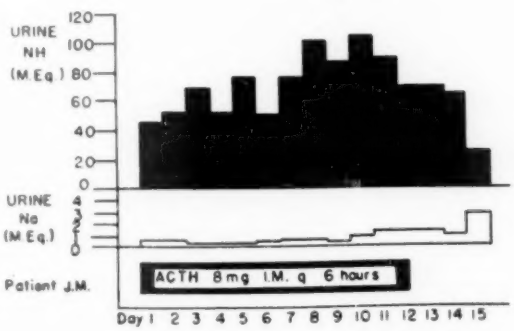


Fig. 5.

TABLE 1.—INCREASE IN URINE AMMONIA EXCRETION DURING ACTH THERAPY

| *Patient* | *Average Daily Urine $NH_3$ Excretion (mEq.) (Control)* | *Average Daily Urine $NH_3$ Excretion (mEq.) (During ACTH)* | *Per Cent of Increase* |
|---|---|---|---|
| B.E. | 41.6 | 67.7 | 62.7 |
| R.E. | 26.3 | 43.2 | 64.2 |
| C.D. | 15.7 | 35.3 | 125.0 |
| V.M. | 9.3 | 44.6 | 380.0 |
| L.D. | 45.7 | 80.6 | 76.3 |
| M.O'L.* | began with 21.7 mEq. | and rose to 64.3 mEq. $NH_3$/day | |
| J.M.* | began with 44.5 mEq. | and rose to 102.0 mEq. $NH_3$/day | |
| L.A.* | began with 19.7 mEq. | and rose to 110.0 mEq. $NH_3$/day | |
| P.T. | 3.8 | 3.2 | —— |
| L.P. | 24.0 | 29.7 | —— |

* Inadequate control period prior to ACTH administration.

TABLE 2.—DATA REPRESENTING PATIENT L.D. (No. 8)

| *Day* | *Urine $NH_3$ (mEq.)* | *Urine pH* | *Urine Titratable Acidity (mEq.)* | *Urine Na (mEq.)* | *Weight (lbs.)* | *ACTH (mg.)* |
|---|---|---|---|---|---|---|
| 1 | 25.5 | —— | 111.0 | 3.0 | 163.2 | —— |
| 2 | 30.4 | —— | 125.0 | 8.2 | 162.1 | —— |
| 3 | 72.2 | —— | 73.7 | 13.2 | 161.8 | —— |
| 4 | 58.0 | —— | 254.0 | 18.0 | 161.9 | —— |
| 5 | 32.6 | —— | 232.0 | 37.8[2] | 161.2 | —— |
| 6 | 52.0 | —— | 207.0 | 8.2 | 160.8 | —— |
| 7 | 58.9 | —— | 304.0 | 7.4 | 160.2 | —— |
| 8 | 36.2 | —— | 185.0 | 58.3[2] | 160.0 | —— |
| | | | *ACTH Therapy Begun* | | | |
| 9 | 60.8 | —— | 154.0 | 3.2 | 159.9 | 25 |
| 10 | 68.2 | 5.82 | 418.0 | 0.9 | 160.8 | 25 |
| 11 | 56.7 | 6.19 | 289.0 | 0.8 | 161.0 | 20 mg. q. 6 hrs. |
| 12 | 59.8 | 6.27 | 263.0 | 0.5 | 161.0 | " " " |
| 13 | 70.0 | 6.46 | 235.0 | 0.5 | 161.6 | " " " |
| 14 | 69.1 | 6.48 | 190.0 | 0.5 | 161.6 | " " " |
| 15 | [1] | [1] | [1] | 0.5 | 162.0 | " " " |
| 16 | 88.3 | 6.20 | 330.0 | 0.4 | 161.8 | " " " |
| 17 | 98.7 | 6.28 | 279.0 | 0.4 | 161.9 | " " " |
| 18 | 100.0 | 6.18 | 359.0 | 0.5 | 161.6 | " " " |
| 19 | 88.6 | —— | 316.0 | 0.5 | 161.9 | " " " |
| 20 | 111.0 | —— | 310.0 | 0.5 | 162.1 | " " " |
| 21 | 85.2 | —— | 192.0 | 12.2 | 161.5 | " " " |
| 22 | 91.0 | —— | 197.0 | 120.0 | 159.8 | " " " |
| | | | *ACTH Therapy Discontinued* | | | |
| 23 | 81.8 | —— | 146.0 | 243.0 | 157.2 | —— |
| 24 | 69.8 | —— | 59.3 | 356.0 | 152.0 | —— |
| 25 | 49.9 | —— | 66.6 | 530.0 | 146.0 | —— |
| 26 | 62.7 | —— | 83.4 | 553.0 | 137.9 | —— |
| 27 | 33.2 | —— | 66.0 | 346.0 | 131.2 | —— |
| 28 | 34.2 | —— | 70.9 | 241.0 | 124.9 | —— |
| 29 | 30.4 | —— | 118.0 | 256.0 | 121.4 | —— |
| 30 | —— | —— | —— | —— | 118.9 | —— |

[1] Specimen collection unsatisfactory
[2] Mercurial diuretic administered

kidneys. His course over the years was progressively worse. In February, 1952, he was admitted in severe edema and uremia. ACTH therapy was unsuccessful and he died during this hospitalization.

CASE 10. Patient L.P. (Fig. 9) is a 24-year-old male who has had known diabetes mellitus since the age of 9. His diabetes was difficult to control. He was seen in December, 1951, in severe edema with poorly controlled diabetes. Kidney damage was severe. He responded well to control of his diabetes and an acid-ash diet. A series of carbuncles preceded his admission in November, 1952. Edema was marked at that time and he was in severe uremia. There was no clinical response to ACTH and he expired during this hospital stay.

**Results.** Ammonia excretion rose significantly in 8 of 10 patients studied (Table 1). This rise was usually of increasing measure during the first few days of ACTH administration (Figs. 1, 2, 3, 4, 6, 7). A precipitous fall in urine ammonia excretion fre-

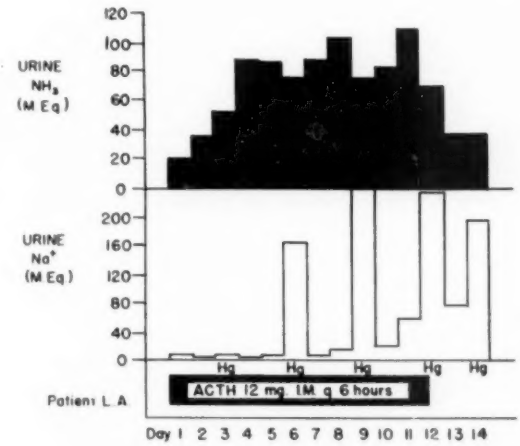


Fig. 6.

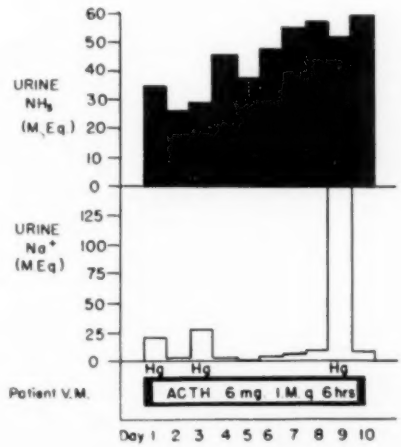


Fig. 7.

quently followed the discontinuance of ACTH therapy (Figs. 1, 2, 3, 5, 6 and Table 2). It might appear that urine sodium and ammonia excretion were inversely related (Figs. 1 and 2). However, ammonia ion excretion continued to rise, remain elevated, or decrease minimally despite a large degree of spontaneous sodium diuresis (Figs. 2, 3 and 4) or sodium diuresis initiated by mercurial diuretics (Figs. 6 and 7). Furthermore, urinary sodium excretion may be sharply decreased during ACTH administration without an associated rise in ammonia (Fig. 8) or only a minimal rise in ammonia excretion (Fig. 9). Urinary sodium and ammonia ion concentration do not seem directly to influence each other in this respect.

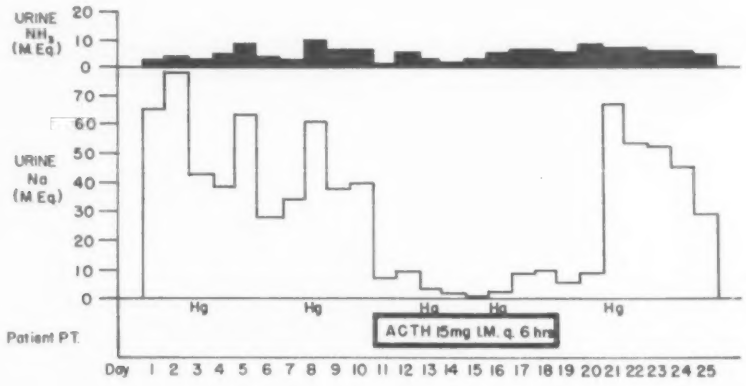


Fig. 8.

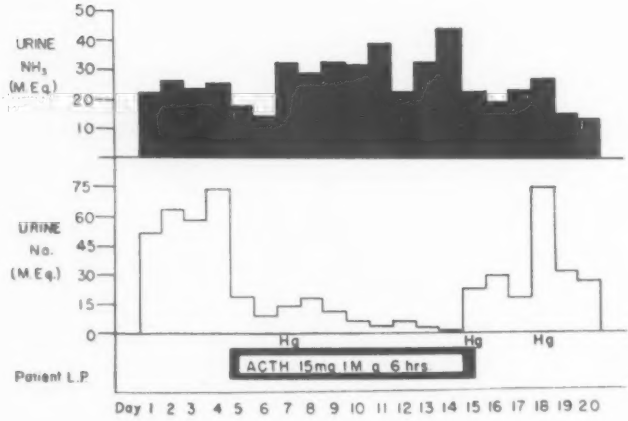


Fig. 9.

Table 2 presents some of the data obtained during the study and therapy of patient L.D. The expected rise in ammonia ion excretion is noted. Titra-

table acidity values also rose during ACTH administration from an average daily excretion of 186 mEq. in the control period to an average of 272 mEq. during ACTH. Urinary pH showed no trend toward the acid side while urinary ammonia excretion was rising.

It can also be noted from these graphic representations that ACTH-induced diuresis occurs in 3 patterns. This has been reported previously[10,11].

1. A spontaneous diuresis in the days following ACTH discontinuance (Figs. 1, 2, 4).

2. A spontaneous diuresis of sodium and water beginning during the period of ACTH administration, usually on the fifth or sixth day (Figs. 2, 3, 4).

3. Diuresis may be delayed until ACTH restores renal tubular "sensitivity" to mercurial diuretics (Figs. 6 and 7).

**Discussion.** It has been established by this study that ammonia production by the tubules of the nephrotic kidney is significantly increased during ACTH administration. Nephrotic patients who responded well to ACTH therapy demonstrated ammonia excretion rises of 62% to 380% as compared to control values obtained before ACTH was administered. The rise in ammonia excretion is indisputable. The mechanism of this rise, however, remains to be determined. Clues as to the possible mechanism involved might be gained by a discussion of some of the known facts concerning ammonia production by the kidneys.

The active ability of the renal cortex to split ammonia from amino acids has been demonstrated by Krebs[16]. This ability resides in the distal tubular cells of the kidney and is principally dependent upon the enzymatic deamination of the amino acid glutamine[28]. Production of this ion is stimulated primarily by decreases in urine pH[4,5]. The importance of the ammonia ion in the maintenance of acid-base balance by conservation of base is well recognized. However, its importance is small in the day to day regulation of this balance. There is, therefore, little correlation between urine pH and 24-hour output of ammonia in normal states[12]. The physiologic significance of this mechanism increases as degrees of acidosis become more severe and of longer duration. Marked changes of urine pH toward the acid side stimulate ammonia formation[4,5]. This stimulation even when of acute degree results in at best a sluggish response[7,8]. The full weight of ammonia ion conservation of base is often not felt until severe acidosis has been present for several days. "Slow to anger," but strong and persistent, characterizes the ammonia production mechanism. Ammonia ion formation is also capable of more rapid responses under special conditions. This is encountered in the sudden rises in urine ammonia due to lactic acid after vigorous exercise or upon rebreathing $CO_2$[9]. It is, therefore, not always a sluggish mechanism. It is fundamentally related to hydrogen ion concentration[18,20].

Hydrogen ions are not the sole regulator of ammonia production. Other factors interplay. The most important of these factors is the role of adrenal corticosteroids. Adrenalectomized rats demonstrate a greatly impaired production of ammonia ions in response to severe stresses in the form of acidosis[21]. This defective response is a reflection of an impaired deamination of amino acids by the renal cortex in these animals[15,19]. Correction of these deficiencies results when adrenal corticosteroids are administered. We may also accept the fact, then, that adequate adrenal cortex function is necessary for adequate ammonia ion production by the tubular cells in response to stress caused by acid-base imbalance.

The significant rise in ammonia ion production encountered in 8 of the 10 patients included in this series (Table 1) would appear to be more than coincidental. Added weight is given this statement when one considers the precipitous fall in ammonia excretion encountered in some when ACTH was suddenly withdrawn (Figs. 1, 2, 3, 5 and Table 2). This is a sudden change in a usually sluggish mechanism. A relationship to ACTH administration can be established by these observations. ACTH or its hormonal associates from the adrenal cortex may act directly on distal tubular cells to produce this increase; or, they may do so as a secondary manifestation of some other action.

Sodium ion diuresis might, upon superficial inspection of the graphic representations presented, appear to enjoy an inverse and causal relationship to ammonia ion concentration (Figs. 1 and 2). However, urinary sodium excretion may be sharply decreased during ACTH therapy without an associated rise in ammonia production (Fig. 8), or only a small rise in ammonia (Fig. 9).* Ammonia production, furthermore, continues to rise, remain elevated, or decrease only minimally despite a decided degree of spontaneous sodium diuresis (Figs. 2, 3 and 4) or sodium diuresis initiated by mercurial diuretics (Figs. 6 and 7). We may reason that these two ions are primarily if not entirely independent relative to the rise in ammonia excretion during ACTH therapy.

Changes of urine pH to the acid side stimulate ammonia production as stated previously. Urine pH showed no changes toward the acid side during the period of increasing ammonia production as studied in patient L.D. (Table 2). Titratable acidity in the same patient did show a tendency to rise during hormone administration (Table 2). The meaning of this rise in relation to ammonia production is not clear. Innumerable factors, many of which are poorly understood, influence the degree of titratable acidity. It is of interest to note that titratable acidity in the urine is, like ammonia, under strong adrenal cortical control[21]. Adequate adrenal cortical function, at least in the rat, is of even greater importance in titratable acidity production than in ammonia formation[21]. Whether the rise in titratable acidity encountered in this patient secondarily caused an increase in ammonia, or whether it was a reflection of hormone action on tubular cells independent of the ammonia mechanism, is not clear. The latter seems the more likely. Urine pH and titratable acidity are two aspects of this problem that need further study.

We arrive, then, at the conclusion that ammonia ion production is increased by the distal tubular cells in patients with the nephrotic syndrome most probably by the direct effect of ACTH or its hormonal associates from the adrenal cortex. These hormones are known to affect several tubular functions. The tubular transport of uric acid and phosphorus is increased by ACTH[14]. The impaired ammonia and hydrogen ion production in adrenalectomized rats, as mentioned previously, is an exhibition of tubular effect. The return of renal "sensitivity" to mercurial diuretics in refractory nephrotic and cardiac patients upon ACTH administration also in all probability demonstrates an effect on tubular enzyme systems[10,11]. It is reasonable to assume that ACTH directly affects the enzyme systems in the tubular cells that are involved in the production of ammonia from amino acids. Another possibility

*It is of additional interest to note the reversal of a salt-losing tendency in these advanced cases of nephrosis when ACTH was administered.

is that increased amounts of the amino acid glutamine are made available to this system which may be deficient in this substance. A hypoaminoacidemia has been reported in nephrotics[6]; also, glutamine is present in decreased amounts in nephritic kidneys[28]. ACTH, in studies done upon patients with rheumatoid arthritis, has the ability to increase the fasting blood level of amino acids[2] and the excretion of amino acids in the urine[13]. However, a rise in blood amino acids does not by itself increase the excretion of ammonia[17]. Therefore, the effect may be manifested in the tubular system.

The clinical significance of this rise in ammonia ion excretion is not clear. Although the rise occurred in all 8 patients in this series who responded well to ACTH therapy and did not occur in the two failures with very advanced degrees of disease, the evidence is insufficient to justify its use as an index of good response.

Our enthusiasm for the use of ACTH in nephrosis remains high, despite recent reports noting unsatisfactory results. We have obtained good results in a majority of patients despite the use of smaller than recommended dosages of ACTH. However, our enthusiasm for ACTH in this disease state does not permit it to supersede the rigid control of patients by the use of an acid-ash, salt-poor diet, adequate fluid intake, adjunctive use of diuretics when indicated, and education of the patient[22-26], before, during, and after the use of this hormone.

**Summary and Conclusions.** 1. ACTH has been successfully employed in the treatment of 8 patients with the nephrotic syndrome of a total of 10 patients included in this series.

2. Urine ammonia excretion rose from 62% to 380% above control values in these 8 patients during the administration of ACTH.

3. The clinical and physiologic significance of this rise in ammonia production is not understood. It seems in all probability to be due either to a direct effect of ACTH or its hormonal associates from the adrenal cortex upon the enzyme system concerned with the deamination of glutamine; or, it may be due to an increased availability of the amino acid glutamine.

The technical assistance of Edward Lustgraff, B.S., and Catherine Reid, B.S., is gratefully acknowledged.

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## SUMMARIO IN INTERLINGUA

### Le Effecto de Corticotrophina (ACTH) Super le Production de Ammoniaco in le Syndrome Nephrotic

1. ACTH esseva usate con successo in le tractamento de 8 ex 10 patientes con le syndrome nephrotic.

2. In iste 8 patientes le excretion urinari de ammoniaco se augmentava durante le administration de ACTH per inter 62 e 380 pro cento supra le valores de controlo.

3. Le signification clinic e physiologic de iste augmento del production de ammoniaco non es clarmente comprendite. Il es multo probabile que illo es o (1) le consequentia de un effecto directe exercite per ACTH o su associatos ab le cortice adrenal super le systema enzymatic concernite con le disamination de glutamina o (2) le resultato del augmentate disponibilitate de glutamina amino-acide.

# THE COMBINED EFFECTS OF CHLOPROMAZINE AND PENTOBARBITAL ON CEREBRAL HEMODYNAMICS AND METABOLISM*

By Joseph F. Fazekas, M.D.

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and

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The potentiation of central nervous system depressants by chlorpromazine was first demonstrated by Laborit *et al.* [8].These findings have subsequently been confirmed and extended by other investigators[12].

The clinical application of such combinations is usually attended by the injunction that dosage of the aliphatic compound may be considerably reduced from the quantities normally administered alone. Although the simultaneous use of chlorpromazine usually permits satisfactory sedation with comparatively small doses of barbiturates, in some instances a more profound hypnosis may be desired, requiring larger amounts of barbiturate. It was considered worthwhile to determine whether the combination of chlorpromazine with relatively large doses of pentobarbital would demonstrably influence cerebral hemodynamics and oxygen consumption.

**Material and Methods.** The subjects of the present study were 7 convalescent hospital patients ranging from 24 to 44 years of age, recovering from a variety of acute illnesses. Pentobarbital was administered intravenously in doses ranging from 250 to 400 mg. over a period of 5 minutes. Electroencephalographic tracings were made at frequent intervals until 30 to 45 minutes following administration of the drug, when quantitative cerebral studies were performed. At the end of the latter procedure, blood specimens were taken for barbiturate estimation. At this time chlorpromazine was administered intravenously within a 5-minute period in doses ranging between 20 and 60 mg. Thirty minutes after the administration of chlorpromazine, cerebral studies and electroencephalograms were repeated.

Scheinberg and Stead's modification[11] of the Kety and Schmidt procedure[6] for the determination of cerebral blood flow (CBF) was applied in this study. The gas mixtures utilized were those described by Kety[6]. Blood oxygen was determined by the manometric technique of Van Slyke and Neill[9]. Mean arterial blood pressure (MAP) was obtained directly from the femoral artery by means of a damped aneroid manometer[1]. The cerebral oxygen consumption ($CMRO_2$) and cerebral vascular resistance (CVR) were calculated as follows: $CMRO_2$ (ml. $O_2$/min./100 gm. brain) $= \frac{CBF \times (A-V)O_2}{100}$, where $(A-V)O_2$ denotes arteriovenous oxygen difference. CVR (mm. Hg/ml. blood/min./100 gm. brain) $= \frac{MAP}{CBF}$ Goldbaum's method[4] was used for the estimation of blood pentobarbital levels.

**Results.** Table 1 presents the findings of these studies. The moderate hypotensive effect of chlorpromazine[10] is demonstrated by the fall in mean ar-

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Chlorpromazine (Thorazine) was supplied by Smith, Kline & French Laboratories, Philadelphia, Pennsylvania.

TABLE 1.—EFFECTS OF CHLORPROMAZINE AND PENTOBARBITAL ON CEREBRAL HEMODYNAMICS AND METABOLISM

| | | Drugs | | | MAP | | CBF | | CVR | | $CMRO_2$ | | Art. $O_2$ | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Subj. | Age (Yrs.) | Pent. (mg.) | Chlor. (mg.) | Pent. Level (mg./L.) | Pent. | Chlor. | Pent. | Chlor. | Pent. | Chlor. | Pent. | Chlor. | Pent. | Chlor. |
| K.J. | 35 | 400 | 60 | 0.5 | 85 | 66 | 30.2 | 34.9 | 2.8 | 1.9 | 2.1 | 2.2 | 13.11 | 11.90 |
| B.G. | 27 | 300 | 50 | 0.4 | 79 | 68 | 54.4 | 43.6 | 1.5 | 1.6 | 2.7 | 1.9 | 12.05 | 11.96 |
| H.R. | 40 | 330 | 20 | 4.0 | 77 | 66 | 47.5 | 40.0 | 1.6 | 1.7 | 3.1 | 2.8 | 14.76 | 14.77 |
| T.J. | 44 | 270 | 40 | 3.5 | 96 | 75 | 46.4 | 37.2 | 2.1 | 2.0 | 2.7 | 2.4 | 16.63 | 16.64 |
| M.J. | 26 | 250 | 42 | 2.5 | 110 | 78 | 50.1 | 43.0 | 2.2 | 1.8 | 2.8 | 2.7 | 14.63 | 14.36 |
| F.L. | 38 | 360 | 54 | 6.0 | 92 | 75 | 39.9 | 32.7 | 2.3 | 2.3 | 2.4 | 1.8 | 12.93 | 12.55 |
| P.H. | 24 | 300 | 45 | 3.9 | 92 | 77 | 33.6 | 38.6 | 2.7 | 2.0 | 3.1 | 2.7 | 18.21 | 18.11 |
| | | | | | | | *Mean Values* | | | | | | | |
| | 33 | 326 | 44 | 3.0 | 90 | 72* | 43.2 | 38.6 | 2.2 | 1.9 | 2.7 | 2.4 | 14.62 | 14.33 |

* Significant difference, $p < .01$.

In this table, **MAP** signifies mean arterial blood pressure in mm. of Hg; **CBF**, cerebral blood flow in ml./100 gm. of brain/min.; **CVR**, cerebral vascular resistance in mm. of Hg/ml. blood/100 gm. of brain/min.; $CMRO_2$, cerebral oxygen consumption in ml./100 gm. of brain/min.; Art. $O_2$, arterial oxygen in vol. %. "Pent." indicates pentobarbital, and "Chlor.", chlorpromazine.

terial blood pressure to an average of 72 mm. Hg from an average value of 90 mm. Hg with barbiturate sedation alone. Chlorpromazine did not interfere with the usual diminution in cerebral vascular resistance (which fell from 2.2 to 1.9) attendant upon blood pressure reduction[3] so that cerebral blood flow remained essentially unchanged. The cerebral metabolic rate showed no significant alteration when chlorpromazine was administered to subjects under the influence of pentobarbital, blood levels of which ranged from 2.5 to 6.0 mg. per 100 ml. The arterial oxygen content was essentially unchanged after administration of chlorpromazine.

Following pentobarbital administration, the electroencephalograms became disorganized and showed both slow and fast high voltage activity. After chlorpromazine, the records were either unchanged or demonstrated better organization, and in a large number of cases typical sleep spindles were noted.

**Discussion.** It has previously been demonstrated that amounts of barbiturate sufficient to produce hypnosis do not demonstrably affect cerebral hemodynamics and oxygen consumption, while larger doses may produce a significant reduction of both measurements[5,7]. In these studies relatively large doses of pentobarbital (up to 400 mg.) were administered along with amounts of chlorpromazine sufficient to produce moderate tranquilization when given alone. Evidently these doses of pentobarbital were sufficiently large to produce some diminution of cerebral blood flow and metabolism, since the values obtained were definitely lower than those usually obtained for unsedated subjects in this age group[2]. In consequence, such values might be considered a reasonably sensitive base line for determining possible synergistic or additive effects of chlorpromazine on these functions. Since no sig-

nificant changes were observed, it seems permissible to conclude that the two drugs in the doses used act predominantly on different areas of the central nervous system, or that chlorpromazine inherently produces negligible interference with cerebral hemodynamics and oxidative reactions. Clinically the tranquilizing influence of chlorpromazine was particularly notable in 3 individuals who had reacted to pentobarbital with marked hyperactivity. In one of these cases (not included in this series), it was impossible to perform initial quantitative studies because of the subject's marked agitation. All the remaining individuals showed increased sedation following the administration of chlorpromazine, and subsequent recovery appeared to be definitely delayed, compared to that following similar amounts of barbiturate alone; however, there was no clinical evidence of respiratory depression, and arterial oxygen content remained essentially unchanged. It would appear from these studies that in the presence of moderately large doses of pentobarbital the concomitant administration of chlorpromazine does not produce an appreciable further decrease of total cerebral metabolic rate and cerebral blood flow, which might well be expected with increased amounts of barbiturate alone sufficient to produce an equivalent degree of central nervous system depression. A combination of the two drugs might therefore prove more satisfactory than chlorpromazine or barbiturates alone for the management of hyper-excitable states in which tranquilization is difficult to achieve.

**Summary and Conclusions.** Cerebral hemodynamics and metabolism and electroencephalograms were studied before and after the administration of relatively large doses of pentobarbital combined with moderate amounts of chlorpromazine. The characteristic electroencephalographic change after barbiturate administration tended to remain unaltered or even to improve following chlorpromazine administration. There was a slight diminution of cerebral blood flow and cerebral oxygen consumption after pentobarbital; no additional interference with cerebral hemodynamics and oxidative reactions was demonstrated when chlorpromazine was also given. Recovery from sedation induced by the combination of the two drugs was somewhat delayed. Excitation by pentobarbital in 3 subjects was suppressed by chlorpromazine. A combination of the two drugs may be the therapy of choice for the management of refractory hyper-excitable states.

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## SUMMARIO IN INTERLINGUA

### Le Combinate Effectos de Chlorpromazina e Pentobarbital Super Hemodynamica e Metabolismo Cerebral

Hemodynamica e metabolismo cerebral e electroencephalogrammas esseva studiate ante e post le administration de relativemente grande doses de pentobarbital in combination con moderate quantitates de chlorpromazina. Le characteristic alteration electroencephalographic post administrationes de barbiturato tendeva a remaner intacte o mesmo a meliorar se post administrationes de chlorpromazina. Il occurreva un leve diminution del fluxo de sanguine cerebral e del consumption cerebral de oxygeno post pentobarbital. Nulle obstruction additional del hemodynamica e del reactiones oxydative del cerebro esseva demonstrabile quando chlorpromazina esseva etiam administrate. Recuperation ab sedation inducite per le combination del duo drogas esseva levemente retardate. Excitation per pentobarbital in 3 subjectos esseva supprimite per chlorpromazina. Un combination del duo drogas es possibilemente le therapia de election in le tractamento de statos refractori de hyper-excitabilitate.

## SUMMARIO IN INTERLINGUA

*(See page 622 for original article)*

### Observationes in Re Certe Viruses que Causa Morbos Exanthematose in Humanos

Es passate in revista certe observationes in re rubeola, varicella, e benigne morbo exanthematose observate in Massachusetts in 1951. Es traciate le passos per que histoculturas de viruses ha devenite un plus efficace methodo in le recognition e propagation de etiologic agentes virusal. Un factor de signification particular in iste respecto es le uso de cellulas susceptibile in le medio cultural pro que le viruses es a monstrar se cytopathogenic. Le uso de antibioticos se ha monstrate del plus alte valor in prevenir contamination del histoculturas. Un altere melioration technic ha essite le introduction, in culturas virusal, de culturas de maligne cellulas human, como parte del processo de trypsinisation de cellulas usate in histoculturas. Es describite le application de tests de neutralisation virusal in histoculturas. Il ha essite possibile isolar le agente etiologic de rubeola, e assi on pote probar directemente le anticorpore protective in globulina human. Isto va forsan resultar in le disveloppamento de un methodo practic pro le induction de immunitate active.

# THE HEART IN PROGRESSIVE MUSCULAR DYSTROPHY*

By Francis Grandell, M.D.

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Since 1883, when Ross[10] reported atrophy of the heart muscle in a 12-year-old boy with progressive muscular dystrophy, occasional reports in the literature have focused attention on the fact that this disease may involve the heart to a marked degree. This is shown grossly by severe scarring and adiposity of the heart muscle. The histological findings have been similar to those present in affected striated muscles elsewhere in the body, with atrophy and hypertrophy of muscle cells, loss of cross striation, fragmentation of muscle fibers and increase of interstitial connective and fatty tissue as the most important features.

In view of the oftentimes extensive myocardial scarring encountered in persons with progressive muscular dystrophy it seemed possible that the ballistocardiograph, as a sensitive indicator of myocardial function, might be of some value in detecting myocardial embarrassment in persons with this disorder.

The present paper is a report on the ballistocardiographic findings in a small series of patients with this disease.

**Material and Method.** Sixteen patients receiving treatment for progressive muscular dystrophy at the Rehabilitation Clinic of the Kings County Hospital, Brooklyn, were selected for this study. Five of the 16 patients were females, ranging in age from 8 to 62 years. The male patients were 12 to 55 years of age. All of the patients were ambulatory, except for one female and 3 males who had been confined to wheel-chairs for periods of time varying from 3 months to 2 years. Progressive muscular dystrophy had become manifest in one female and 5 males before the age of 8 years. In all the remaining patients the disease had developed in the second decade of life or later.

None of the patients had a history of having had rheumatic fever. On physical examination no patient showed evidence of cardiac failure. Mild hypertension was present in 5 patients. A moderately loud, early systolic murmur, with point of maximum intensity at the base of the heart, was found in a 62-year-old, hypertensive female, who complained of palpitation and dyspnea on exertion. In a 20-year-old female with a history of palpitation and fatigue following moderate exertion, the cardiovascular findings were normal, except for the presence of tachycardia. Tachycardia was also noted in a 12-year-old, obese boy, who experienced mild dyspnea on exertion. All the remaining patients were symptomatically free of cardiac abnormalities.

The electrocardiogram showed atrioventricular conduction times and QRS intervals of normal duration in all 16 patients. Sinus tachycardia, with rates varying between 95 and 110 beats per minute, was present in 5 patients. Occasional premature atrial contractions were recorded in a 50-year-old hypertensive female and in a 52-year-old, normotensive male with evidence of mild aortic stenosis and insufficiency. In 10 patients QRS complexes with amplitudes in excess of accepted maximal values were recorded in one or more precordial leads $V_1$ to $V_4$[14].

A four-channel direct writer electrocardiograph was used to record a diagnostic ballistocardiogram in three planes simultaneously. A portable electromagnetic pick-up[6], placed across the shins, was used to record motion in the head-foot plane. To record motion in the lateral and dorsoventral planes, a spring-mounted, high frequency table with attached electromagnetic pick-up was placed under the shoulders[5]. In 2 patients with flexion contractures of the knees (Cases 1 and 7), the longitudinal trace was obtained by means

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TABLE 1.—TABLE SHOWING AGE DISTRIBUTION, DURATION OF MANIFEST SKELETAL MUSCULAR INVOLVEMENT, AMPLITUDE OF QRS COMPLEXES IN PRECORDIAL LEADS $V_1$ TO $V_6$ IN ELECTROCARDIOGRAM (A = QRS IN EXCESS OF ACCEPTED NORMAL VALUES), AND BALLISTOCARDIOGRAPHIC FINDINGS (N = NORMAL, A = ABNORMAL) IN 16 PATIENTS WITH PROGRESSIVE MUSCULAR DYSTROPHY (PMD).

| | *Females* | | | | | *Males* | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *R.D.* | *G.L.* | *R.M.* | *A.G.* | *A.H.* | *W.M.* | *J.D.* | *R.J.* | *G.C.* | *J.B.* | *G.B.* | *H.L.* | *E.S.* | *M.M.* | *A.L.* | *J.R.* |
| Case No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
| Age | 8 | 20 | 44 | 50 | 62 | 12 | 12 | 13 | 18 | 23 | 33 | 39 | 43 | 43 | 52 | 55 |
| Duration of Manifest PMD | 1 | 8 | 12 | 10 | 10 | 10 | 9 | 6 | 18 | 17 | 18 | 18 | 24 | 10 | 17 | 16 |
| ECG (QRS amplitude in Leads $V_1$ to $V_6$) | N | A | A | A | N | N | A | A | N | A | A | N | N | A | A | A |
| BCG | N | N | N | A | A | A | N | A | N | A | A | N | N | A | A | N |

of an electromagnetic pick-up, mounted on springs, firmly pressed against the shoulders. For timing purposes a limb lead of the electrocardiogram was inscribed simultaneously. Traces were taken with the patient breathing quietly and with the breath suspended in inspiration and expiration.

**Results.** Normal ballistocardiograms were obtained in 8 patients (Cases 1, 2, 3, 7, 9, 12, 13 and 16).

In a 12-year-old, obese boy (Case 6) abnormally small systolic complexes were recorded in all three planes. The largest IJ measured 1.0 mv. in the longitudinal trace with the patient breathing quietly. With respiration suspended, large N waves were recorded in the head-foot trace.

Large systolic complexes were recorded in the longitudinal and lateral traces in a 13-year-old boy (Case 8). The largest IJ measured 2.7 mv. in the longitudinal trace during normal breathing and increased to 5.0 mv. with the breath held in inspiration. With the breath held in expiration large diastolic and rightward presystolic waves were recorded in the lateral trace.

A 23-year-old normotensive male (Case 10) with a heart rate of 85 per minute, showed a grade I respiratory variation of the systolic complexes with large H and N waves in the longitudinal trace. During held inspiration occasional fused H and J waves were recorded. Digitalization occasioned no change in heart rate nor in the ballistic pattern recorded during normal respiration, nor was there any change in the longitudinal systolic complexes during held expiration after digitalis. However, in held inspiration the head-foot trace showed much larger J waves following digitalis.

A grade I respiratory variation of the systolic complexes with large H waves was obtained in the longitudinal trace in a 43-year-old, hypertensive male (Case 14). The largest IJ in the head-foot plane measured 0.9 mv. and in the

lateral plane 1.6 mv. with the patient breathing quietly.

A grade II respiratory pattern was recorded in 2 hypertensive patients, of whom one, a 50-year-old female (Case 4) showed relatively large I waves, small notched J waves and large H and N waves in the longitudinal trace. The second patient (Case 11), a 33-year-old male with a heart rate of 100 beats per minute, showed fusion of the longitudinal H and J waves during normal breathing. With the patient attempting to hold his breath a chaotic pattern was recorded in all three planes. Following digitalization the heart rate slowed to 85 beats per minute and the longitudinal trace showed normal complexes with the breath held in expiration. Fused H and J and deep K waves were recorded in held inspiration and during normal breathing in the head-foot trace, which remained grade II. Sound traces taken at this time showed no accentuation of the pulmonic second sound.

Presystolic gallop waves were noted in the longitudinal trace in a 62-year-old, hypertensive female (Case 5), who also showed a grade I respiratory variation of the systolic complexes. In a 52-year-old male (Case 15) with mild aortic stenosis and insufficiency, presystolic gallop waves were recorded in the dorsoventral trace.

COMMENT. Information in the literature concerning the incidence of dystrophic cardiac involvement in persons with progressive muscular dystrophy is scant. In one published series[8], 6 of 12 cases coming to necropsy showed myocardial lesions. Zatuchni and co-workers[17], on the basis of an extensive survey of the literature, were able to find 94 cases showing various cardiovascular abnormalities among 136 patients with progressive muscular dystrophy, on whom sufficient data were available to warrant a diagnosis.

Although no relationship between duration and severity of skeletal muscular involvement and those of the heart has been noted[8,16], it seems that, in general, all cases of progressive muscular dystrophy with unequivocal dystrophic cardiac involvement reported in the literature have been of the pseudohypertrophic or childhood type, with onset of skeletal muscular symptoms in early childhood. In one instance, however, myocardial lesions have been observed in a 48-year-old male, who developed overt signs of progressive muscular dystrophy at the age of 29 years[8].

Cases of congestive heart failure responding to digitalis have been observed in a number of instances[1,2,7,11,13,16,17]. However, death in congestive failure has apparently been rare and has been reported by only a few authors[15].

Comprehensive reviews of electrocardiographic findings in persons with progressive muscular dystrophy reported in the literature have been published by Zatuchni and co-workers[17] and by Rubin and Buchberg[11]. The abnormalities have consisted of sinus and ventricular tachycardia, atrial and ventricular extrasystoles, large P waves in the standard limb leads, prolongation of the PR interval, lengthening of the QT interval, shortening of the ST segment, Q waves in leads II and III and various aberrations of the R wave in leads I and II.

In their own series of patients Rubin and Buchberg noted the presence of a short PR interval in 5 patients, of whom two showed deep Q waves in leads I and $aV_L$ and in the second, third and fourth intercostal spaces in the $V_5$ position in one patient, and in leads $V_4$ to $V_6$ in the second patient, who also showed an isoelectric T in leads I and $aV_L$ with a negative T in leads $V_4$ to $V_6$. Of two hypertensive patients

one showed a negative T wave in $CF_5$, and in a second patient a diphasic T was recorded in lead I.

In a large series of patients studied by Weisenfeld and Messinger[16] 50% showed tachycardia, and in 13 of 18 patients with complete precordial leads, large R waves were recorded in leads $V_1$ and $V_2$. Other electrocardiographic abnormalities noted by these investigators consisted of abnormal P waves and similarity of leads $aV_R$ and $aV_L$.

Schott and co-workers[12] have observed the presence of large precordial QRS complexes in patients with the pseudohypertrophic or childhood type of disease and have suggested this finding as a further means of differentiation between the childhood type and the facioscapulohumeral type of progressive muscular dystrophy[9].

In the present series, 7 of 10 patients with a history of onset of progressive muscular dystrophy in the second decade or later showed large QRS complexes in one or more of precordial leads $V_1$ to $V_4$. Other electrocardiographic abnormalities noted in this group were isoelectric T waves in the standard and in the augmented limb leads with inverted T in leads $V_3$ to $V_6$ in one patient, and inverted T in $L_1$, $aV_L$, $V_5$ and $V_6$ in a second patient; both patients, however, were hypertensive. Premature atrial contractions were recorded in a hypertensive female, who also showed marked sinus arrhythmia, and in a male with aortic valvular disease, who showed depressed ST segments in $L_1$, $aV_L$ and $V_2$ to $V_6$, isoelectric T in $L_2$ and $aV_F$, and inverted T waves in $L_1$, $aV_L$ and leads $V_3$ to $V_6$. Diphasic T waves in $L_1$ and $aV_L$, and large R waves in $V_2$ and $V_3$ were recorded in a normotensive 55-year-old male in whom marked deterioration of the ballistocardiogram following smoking was indicative of coronary artery disease[3].

Of 6 patients in whom progressive muscular dystrophy had become manifest at the age of 8 years or earlier, three showed large R waves in one or more of leads $V_1$ to $V_4$. Except for sinus tachycardia in 3 patients, and left axis deviation in one patient with clinical evidence of patent interventricular septal defect, no other electrocardiographic abnormalities were observed in this group.

Ballistocardiographic abnormalities were obtained in 6 patients who showed QRS complexes of large amplitude in one or more precordial leads. In one instance congenital heart disease could account for the very large ballistic complexes recorded in an asymptomatic 13-year-old boy, in whom large diastolic and presystolic gallop waves in the lateral trace were indicative of accelerated cardiac filling, and the physical and electrocardiographic findings were those of ventricular septal defect. In 4 patients ballistocardiographic evidence of myocardial dysfunction could be attributed to aortic valvular disease in one instance and to hypertensive heart disease in the remaining patients. Likewise, hypertensive heart disease could account for the grade I respiratory pattern and presystolic gallop waves recorded in the ballistocardiogram of a 62-year-old female with QRS complexes of normal amplitude in the precordial V leads. However, the effects of digitalization in a 33-year-old hypertensive male seemed to indicate the presence of right ventricular embarrassment, evidenced by the finding of normal systolic complexes in held expiration, with fusion of H and J waves and a grade II respiratory variation of the systolic complexes in the head-foot trace during normal breathing. The absence of an accentuated pulmonic second sound seemed to rule

out any significant increase in pulmonic arterial pressure as the cause of the ballistic abnormality. The electrocardiogram was normal except for tachycardia and large R waves in leads $V_1$ to $V_3$. These findings seemed to indicate right ventricular damage, presumptively due to dystrophic involvement.

In the absence of other clinical findings, it seemed probable that the respiratory variation and large diastolic waves noted in the ballistocardiogram of a 23-year-old, chair-ridden male with large R waves in lead $V_4$ of the electrocardiogram, were due to dystrophic cardiac involvement, although pooling of blood in poorly functioning skeletal muscles with concomitant diminished venous return to the heart could be a contributing factor[4].

The small ballistocardiographic complexes of normal configuration recorded in a 12-year-old boy, who showed precordial QRS complexes of normal amplitude in the electrocardiogram, could be due to the marked obesity of the patient.

**Summary and Conclusions.** A ballistocardiographic study of 16 patients with progressive muscular dystrophy revealed abnormal ballistic patterns in 8 patients. Ten patients gave a history of onset of progressive muscular dystrophy after the age of 10 years, and in this group 5 patients showed ballistic abnormalities which could be attributed to hypertension or valvular heart disease. In this group one patient with ballistocardiographic evidence of right ventricular embarrassment showed large R waves in precordial leads $V_1$, $V_2$ and $V_3$ in the electrocardiogram, a finding suggestive of the childhood type of progressive muscular dystrophy, in which dystrophic involvement of the heart is known to occur.

In a group of 6 patients, in whom manifestations of progressive muscular dystrophy had developed before the age of 8 years, abnormal ballistic traces were obtained in three instances. In one patient the ballistic abnormality could be attributed to myocardial dysfunction, presumptively due to dystrophic involvement. In the remaining 2 patients the abnormal ballistocardiograms were attributed to other causes.

The findings in this small series of cases seem to indicate, that dystrophic involvement of the heart is uncommon, or if present, apparently does not seriously affect myocardial function in the vast majority of patients with progressive muscular dystrophy until late in the course of the disease.

In patients with the facioscapulohumeral type of progressive muscular dystrophy, ballistocardiographic evidence of myocardial dysfunction usually can be attributed to heart disease due to other causes.

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## SUMMARIO IN INTERLINGUA

### Le Corde in Progressive Dystrophia Muscular

Un studio ballistocardiographic de 16 patientes con progressive dystrophia muscular revelava anormal configurationes ballistic in 8 casos. Dece patientes reportava le declaration del progressive dystrophia muscular como habente occurrite post le etate de 10 annos, e in iste gruppo 5 patientes monstrava anormalitates ballistic que poteva attribuer se a hypertension o morbo cardiac valvular. In iste mesme gruppo, un patiente con indicios ballistocardiographic de embarasso dextero-ventricular monstrava large undas R in precordial derivationes $V_1$, $V_2$, e $V_3$ del electrocardiogramma. Iste constatation suggere le typo juvenil de progressive dystrophia muscular in que le affection del corde es un occurrentia cognoscite.

In un gruppo de 6 patientes, in qui manifestationes de progressive dystrophia muscular habeva comenciate disveloppar se ante le etate de 8 annos, anormal registrationes ballistic esseva obtenite in tres casos. In un de iste patientes le anormalitate ballistic poteva attribuer se a dysfunctionamento myocardiac secundari presumptivemente a un involvimento dystrophic. In le remanente duo casos le anormalitates ballistocardiographic esseva attribuite a altere causas.

Le constatationes in iste parve serie de casos pare indicar que affection dystrophic del corde es incommun e, si presente, non apte a seriemente obstruer le functionamento myocardiac in le vaste majoritate del patientes con progressive dystrophia muscular usque multo tarde in le curso del morbo.

In patientes con progressive dystrophia muscular del typo facio-scapulo-humeral, signos ballistocardiographic de dysfunctionamento myocardiac es usualmente attribuibile a morbo cardiac ab altere causas.

# VISCOSITY OF THE BLOOD *IN VITRO* AT VARIOUS TEMPERATURES IN 26 PATIENTS WITH RAYNAUD'S PHENOMENON*

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The following mechanisms are known to be involved in the pathogenesis of the various forms of Raynaud's phenomenon[11]: 1) an abnormally strong vasoconstriction impulse, mediated through the nerves; 2) abnormal susceptibility to cold of the vessel wall; 3) thickening of the vessel wall; 4) narrowing of the supplying artery (for example, the subclavian artery); 5) reversible cold embolism; a) cold agglutination of the erythrocytes; b) cold precipitation of the plasma proteins, that is, cryoglobulinemia.

Abnormalities in the vessel wall (1-4) are generally considered to be more often involved than changes in the blood (5) but there is no quantitative information available on the relative frequency of these mechanisms. A number of reports has appeared describing individuals in whom cold hemagglutination caused acrogangrene[20,24,28,29], Raynaud's syndrome[1,3,4-8,16,18,21,23,27] or acrocyanosis[9,10,13,25]. Likewise, cryoglobulinemia with peripheral vascular symptoms has been described by several authors[2,12,19,26,30]. This paper presents the result of a systematic investigation in the viscosity of blood *in vitro* at various temperatures, in a group of 26 patients with Raynaud's phenomenon.

**Materials and Methods.** The viscosity of blood *in vitro* was measured in a control group of 24 normal adults and in 26 unselected patients with Raynaud's phenomenon. In 6 of these patients local gangrene had occurred. None of the patients or the normal subjects had anemia.

Blood was drawn by vein puncture without resort to tourniquet since stasis has been shown to increase the viscosity[17]. Potassium oxalate (0.5 ml. of a 30% solution for 49.5 ml. of blood) was used as an anticoagulant. This substance does not interfere with the viscosity of plasma[22].

Höppler's eccentrically-falling ball viscosimeter[14,15] was used for the measurements because it permits an accuracy of ± 1%, is easily controlled as to temperature, and is easy to clean. The viscosity was determined from the falling time of the ball over a distance of 50 mm., using the equation $\eta = F \times (S_k - S_f) \times K$, where $\eta$ = absolute viscosity in centiPoises, F = fall-time in seconds, $S_k$ = specific weight of ball, $S_f$ = specific weight of fluid, and K = ball constant. The viscosity was measured at temperatures between 5° and 40° C., making duplicate readings at each 10° interval. A ball giving at least 50 seconds of fall time was chosen. Above this limit there is no danger of turbulence. No thixotropy or dilatancy was seen, that is, predictable measurements were possible even using balls of various diameters.

**Results.** The viscosity of the blood at body temperature in both the control group and the patients fell in the range of 3 to 4 centiPoises with no significant differences between the two groups. The following three figures were arbitrarily chosen as indicators of the influence of cold upon the blood viscosity: 1) the fall in temperature necessary to increase the viscosity from

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a level of 4 to one of 5 centiPoises; 2) the fall in temperature necessary to increase the viscosity from a level of 8 to one of 10 centiPoises; and 3) the viscosity in centiPoises at 8° C.

These figures appear in Table 1. It can be seen 1) that the decrease in temperature necessary for raising the blood viscosity was the same for the patients' blood as for normal blood, both when the blood was warm (viscosity range 4 to 5 cP) and when it was cold (8 to 10 cP), and 2) that there was no difference in the viscosity at 8° C. between the control group and the patient group. These results applied to all patients but one (shown in brackets in the table).

This patient, a 52-year-old female, suffered from chronic lymphocytic leukemia with a history of several years of typical angiospastic attacks. Her blood, when cooled below 26° C., turned into a solid mass. Her plasma, obtained by centrifugation of blood at body temperature, showed normal viscosity at all temperatures measured. The cold hemagglutination titer of the patient's blood was found to be 512 (1024).

TABLE 1.—THE INFLUENCE OF COLD ON THE VISCOSITY OF THE BLOOD IN 9 NORMAL PERSONS AND 26 PATIENTS WITH RAYNAUD'S PHENOMENON

| | 1* °C. | | 2** °C. | | 3*** cP | |
|---|---|---|---|---|---|---|
| | *Controls* | *Patients* | *Controls* | *Patients* | *Controls* | *Patients* |
| | 4.9 | 5.6 | 3.2 | 3.0 | 8.0 | 6.9 |
| | 6.6 | 5.8 | 3.6 | 3.6 | 8.1 | 7.3 |
| | 7.2 | 6.0 | 4.0 | 3.7 | 8.2 | 7.4 |
| | 7.4 | 6.3 | 4.5 | 3.8 | 8.5 | 7.6 |
| | 7.5 | 7.0 | 4.8 | 3.9 | 8.8 | 7.6 |
| | 8.0 | 7.3 | 5.0 | 4.0 | 9.7 | 8.0 |
| | 8.4 | 7.5 | 5.1 | 4.1 | 10.1 | 8.0 |
| | 8.4 | 7.6 | 5.5 | 4.3 | 11.2 | 8.0 |
| | 8.8 | 7.8 | 5.7 | 4.3 | 11.2 | 8.2 |
| | | 8.0 | | 4.4 | | 8.5 |
| | | 8.3 | | 4.4 | | 8.6 |
| | | 8.3 | | 4.6 | | 8.7 |
| | | 8.4 | | 4.6 | | 8.8 |
| | | 8.4 | | 4.8 | | 9.0 |
| | | 8.5 | | 5.0 | | 9.0 |
| | | 8.7 | | 5.0 | | 9.1 |
| | | 8.8 | | 5.0 | | 9.2 |
| | | 9.0 | | 5.0 | | 9.3 |
| | | 9.2 | | 5.1 | | 9.3 |
| | | 9.2 | | 5.2 | | 9.5 |
| | | 9.2 | | 5.4 | | 9.6 |
| | | 9.2 | | 6.1 | | 9.7 |
| | | 9.3 | | 6.3 | | 10.0 |
| | | 10.4 | | 6.4 | | 10.0 |
| | | 13.5 | | 6.5 | | 11.0 |
| | | (13.0) | | (solid) | | (solid) |
| Mean | 7.47 | 8.29 | 4.60 | 4.74 | 9.31 | 8.73 |
| St. Dev. | ±1.12 | ±1.57 | ±0.85 | ±0.89 | ±1.30 | ±1.05 |
| | No significant difference between the two groups by a "t" test. | | No significant difference between the two groups by a "t" test. | | No significant difference between the two groups by a "t" test. | |

* The fall in temperature necessary to increase the viscosity from 4 to 5 centiPoises.
** The fall in temperature necessary to increase the viscosity from 8 to 10 centiPoises.
*** The viscosity in centiPoises at 8° C.
In brackets the values for a patient with cold hemagglutination.

**Discussion.** The fact that the periph-

eral vascular symptoms in only one of the 26 patients studied could be attributed to reversible cold embolism shows that this mechanism is a rare causal factor in Raynaud's phenomenon.

Measurement of the viscosity of blood *in vitro* is a very easy procedure. Besides the viscosimeter used in the present study, several even simpler models are available with temperature regulation. A determination of the viscosity of whole blood at low temperatures will reveal both types of reversible cold embolism. Cold hemagglutination as well as cryoglobulinemia will cause a marked increase in the viscosity. Plasma, separated by routine procedures, is not suited for the measurement since cryoglobulins may be precipitated at low room temperatures and thus remain hidden in the sediment of erythrocytes[12]. If an abnormal viscosity of whole blood is found at low temperatures, a repetition of the viscosity determination, using plasma separated by centrifugation at body temperature, will enable one to differentiate between the two mechanisms of reversible cold embolism. Cryoglobulinemia will cause abnormal viscosity by both procedures, whereas cold hemagglutination will have no effect on the viscosity of plasma.

Because of the simplicity of the test, it is suggested that a determination of the viscosity of whole blood *in vitro* at low temperatures be included in the examination of patients with Raynaud's phenomenon.

**Summary.** In 26 patients with Raynaud's phenomenon the viscosity of blood was measured *in vitro* over a temperature range of from 5° to 40° C. The blood from only one patient showed abnormally high viscosity in cold, the mechanism being one of cold hemagglutination.

Reversible cold embolism, that is, intravascular clogging of the blood in cold, caused by cold hemagglutination or solidifying of cryoglobulins, therefore seems to be a rare causal factor in the mechanism of the Raynaud's phenomenon.

A measurement of the viscosity of blood *in vitro* at low temperature (for example, 8° C.) is a very simple procedure, which will detect both types of cold embolism. It is suggested that this test should be included in the examination of patients with Raynaud's phenomenon.

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## SUMMARIO IN INTERLINGUA

### Viscositate del Sanguine *in Vitro* a Varie Temperaturas in 26 Patientes con le Phenomeno de Raynaud

In 26 patientes con le phenomeno de Raynaud le viscositate del sanguine esseva mesurate *in vitro* a varie temperaturas inter 5 e 40 C. Le sanguine de un sol patiente monstrava un anormalmente alte viscositate a basse temperaturas. Le mechanismo in iste caso esseva cryohemagglutination.

Reversibile cryoembolismos, *i.e.* coagulos intravascular a basse temperaturas causate per cryohemagglutination o solidification de cryoglobulinas, pare ergo entrar rarmente como factor causal in le mechanismo del phenomeno de Raynaud.

Le mesuration del viscositate de sanguine *in vitro* a basse temperaturas (per exemplo a 8 c) es un simplissime procedimento que detege ambe typos de cryoembolismo. Nos propone que iste test merita esser includite in le examine de patientes con phenomeno de Raynaud.

# PROGRESS
# OF
# MEDICAL SCIENCE

PEDIATRICS

UNDER THE CHARGE OF

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## THE TURNOVER AND CIRCULATION OF THE PLASMA PROTEINS IN THE BODY

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"AMONG the phenomena which the operations of organic life are constantly presenting to our view, none seem more calculated to excite curiosity than those which attend the formation and uses of the lymph." Such was the introduction of James Moultrie to his discussion of the interaction of lymph, chyle and blood in the first volume of this journal in 1827[145], and no more enthusiastic thought could initiate the present report. The purpose of this paper is to consider briefly, in the light of present concepts, the turnover of certain plasma proteins of more general clinical interest, and their circulation through other compartments of the body.

The plasma proteins are responsible for a broad range of physiological functions, including transportation of metabolites, osmotic equilibrium, enzymatic controls and immunity, to mention a few. As yet very little is understood about the true life history of the individual proteins responsible for these activities.

Since the initial studies by the Schoenheimer group[177], dating from 1939, the dynamic state of body constituents has been recognized and increasing efforts have been made to investigate the fate of plasma proteins in humans. The quantity of circulating proteins is dependent upon a carefully

*Playtex Park Research Institute Grantee.

balanced regulation of the respective rates of synthesis and degradation. The usefulness of any one of the proteins of plasma will be a function not only of the structural characteristics which are adapted to a special purpose, but also of the manner and efficiency by which the protein is delivered to the specific sites at which it reacts. Thus, a variety of pathological states, such as agammaglobulinemia, Wilson's disease, hypoalbuminemia, have been ascribed to primary or secondary changes in the availability of these entities.

The sequential alterations of protein metabolism in disease have been reflected for some years by simple chemical fractionation of plasma proteins into albumin and globulin components. Newer knowledge of the special functions and more precise measurements of different plasma proteins have made more comprehensible the interpretation of alterations.

The ensuing discussion is oriented, therefore, to a general picture of the circulating proteins with special emphasis placed upon 1) problems of identifying proteins during dynamic studies, 2) the turnover of plasma proteins and certain alterations associated with clinical syndromes, and 3) the route of circulation of proteins through the body compartments and some attending aberrations.

**Identification of Plasma Proteins and Methods of Turnover Studies.** Although alterations in the turnover of proteins occur frequently in childhood disease, for example, malnutrition, nephrosis, liver disease, and others, little investigation applying more specialized techniques has been done. Consequently interpretations of the behavior of proteins in the young age group must be inferred, in many instances, from comparable situations studied in adults and animals.

For purposes of identification or quantitation of individual plasma proteins, a variety of fractionation schemes have been employed singly or in combination. Thus "salting out" procedures with sulfates and phosphates[44,85], the specific effects produced by certain heavy metals (for example, zinc, mercury, barium) and organic solvents (for example, ethanol, acetone)[37], electrophoresis in free solution[129] or in solid supporting media (paper, starch, gels)[49,113], analytical and preparative separations in the ultracentrifuge[159], have formed the basis of many studies. To these must be added the advantages of measuring specific enzymatic or chemical activity of certain proteins. The usefulness of immunological specificity for the identification of proteins has been enhanced by the development of such new methods as gel diffusion analysis by Oudin[155], Ouchterlony[152] and Grabar and Williams[81,214]. In addition, the application by Coons and Kaplan[41] of an immunohistochemical technique, utilizing antibody conjugated with fluorescine isocyanate, has made possible direct visual identification of specific protein types in microscopic tissue sections. The extensive consideration of all of these methods elsewhere precludes the necessity of further enlargement here.

The descriptive terminology applied to the components of plasma proteins defined by electrophoresis (pH 8.6, $\Gamma/2$ 0.1) has become the arbitrary standard of reference for comparing the components of other fractionation methods (albumin, $\alpha_1$-, $\alpha_2$-, $\beta$-, fibrinogen, $\gamma$-globulins)[47]. Since the components and their constituent proteins separated by the above methods may not be directly comparable, some confusion in nomenclature has arisen. Thus an elevation of lipoprotein in the serum may be characterized as an $\alpha_1$- or $\alpha_2$-globulin when separated by means of preparative zone electrophoresis[112] but

TABLE 1.—COMPARISON OF TERMS APPLIED TO SOME PLASMA PROTEIN FRACTIONS DERIVED BY DIFFERENT PROCEDURES.

| | *Electrophoretic Property** | | | | *Salt Fraction ($Na_2SO_4$)* | |
|---|---|---|---|---|---|---|
| *Plasma Proteins* | *Component* | *Conc. Per Cent* | *Mobility $\mu \times 10^{-5}$* | *Ethanol Fraction* | *Milne*[131] | *Kibrick*[107] |
| albumins | albumin | 60 | 5.9 | V | filtrate from 26.8% | filtrate from 27.20% |
| $\alpha_1$-mucoprotein<br>$\alpha_1$-lipoprotein | $\alpha_1$-globulin | 4.6 | 5.1 | IV + V | 26.8% precipitate | |
| $\alpha_2$-lipoprotein<br>$\alpha_2$-mucoprotein<br>ceruloplasmin | $\alpha_2$-globulin | 7.2 | 4.1 | IV + V<br>I + III | pseudoglobulin ($\alpha_1 + \alpha_2$ globulins) | 27.20% precipitate ($\alpha$-globulin) |
| transferrin<br>$\beta$-lipoprotein<br>prothrombin | $\beta$-globulin | 12.1 | 2.8–3.5 | IV + V<br>I + III | | 19.90% precipitate ($\beta$-globulin) |
| fibrinogen<br>isohemagglutinins<br>antihemophilic-globulin | fibrinogen<br>$\gamma_1$ globulin | 5.1 | 2.1 | I + III | 19.6% precipitate<br>euglobulin ($\gamma + \beta$ globulins) | 15.75% precipitate ($\gamma$-globulin) |
| antibodies | $\gamma_2$-globulin | 11.0 | 1.0 | II | | |

Adapted from Hughes[99], Lever[121] and Cohn[36].
*Electrophoresis done in veronal buffer pH 8.6, $\Gamma/2$ 0.1.

The plasma proteins listed in the column on the left and the ethanol fractions do not necessarily coincide with the constituents of the salt precipitation fractions. The electrophoretic classification is the arbitrary standard of reference.

appear as a $\beta$-lipoprotein by means of the ethanol fractionation[12]. Table 1 compares some of the plasma proteins defined by three procedures in common use, and emphasizes the problem of correlating different methods of protein separation.

The use of isotopic labels for tracing the pathways and fate of proteins with-

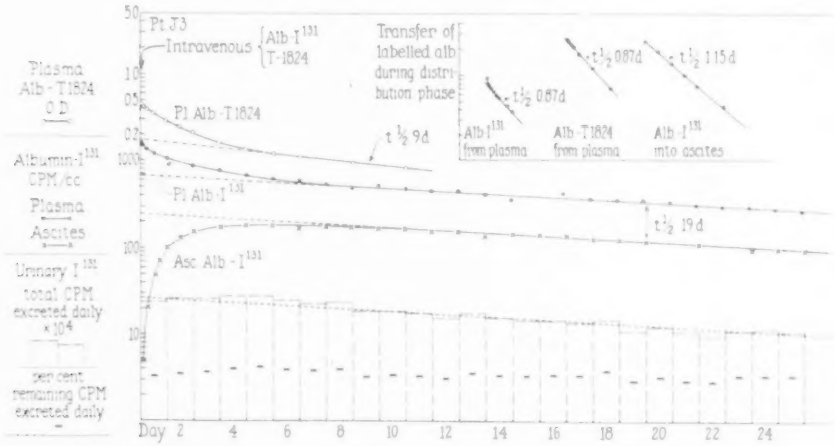


Fig. 1.—Equilibration of albumin $I^{131}$ compared to albumin T1824 following intravenous administration in patient with cirrhosis of liver and ascites[56]. In initial period of 4 to 5 days, transfer of labeled albumin out of blood into extravascular compartments was reflected by simultaneous increase in ascitic fluid values. Decay curves of this distribution phase plotted in inset indicate that rate of transfer of albumin $I^{131}$ and albumin T1824 out of blood was identical ($t_{\frac{1}{2}} = 0.87$ day). Uptake into ascitic fluid was slower.

Following equilibration, labeled protein disappeared during second phase in exponential manner, corresponding to rate of labeled protein degradation. Parallel decrease in plasma and ascitic fluid values indicated complete and continuing equilibration. Degradation rate of albumin T1824 was more rapid than albumin $I^{131}$. Parallel fall of urinary free $I^{131}$ value indicated stable handling of albumin $I^{131}$, 4% degraded daily. Label T1824 was satisfactory for measuring rate of albumin transfer out of blood but less stable than $I^{131}$ for assessing metabolic degradation rate.

in the body has had very restricted use in children because of the lack of precise knowledge regarding tracer dosage limits, isotope distribution in the body and possible sequelae of radiation. In adults, the tracer methods have been biosynthetic incorporation into proteins of $N^{15}$, and radioactive $S^{35}$, $Fe^{59}$, $P^{32}$ and $Cu^{64}$ as well as chemical extrinsic labeling of protein with radiomin $I^{131}$ injected intravenously into a patient with cirrhosis of the liver and ascites. The equilibration of labeled albumin between plasma and ascitic fluid was readily defined and the urinary excretion rates of $I^{131}$ reflected a well stabilized handling of albumin $I^{131}$. In one study[54,56] no difference in this general picture was noted in patients as young as 10 years

compared to those as old as 70 years.

Despite the well ordered turnover of albumin $I^{131}$ reflected in Fig. 1, some problems have become apparent in comparative studies with different isotopic labels which often preclude a satisfactory interpretation of the experimental data. These were considered in some detail recently by Volweiler[205] and Luck[129a]. Thus, the turnover of circulating proteins should always be criticized in the light of the following limitations:

1) *Stability of the Isotopic Label.* The problem of extrinsic isotopic labels $I^{131}$, $Cr^{51}$, (or T1824° in the case of albumin studies) is the relative stability of the isotope-protein bond and whether degradation of this bond coincides with degradation of the protein as a functional unit. Studies with albumin $I^{131}$ [29,54,56,194,195,205] and γ-globulin $I^{131}$ [13,57,91] in different laboratories have given reasonably reproducible findings. Furthermore Sterling[194], has reported identical handling of native human albumin and albumin $I^{131}$ by the rabbit. Similar studies have born out the reliability of the γ-globulin $I^{131}$ bond[57], nevertheless identical behavior of native and $I^{131}$ labeled proteins in the human cannot be assumed. In comparable examinations the extrinsic label $Cr^{51}$ was reported unsatisfactory by Sterling[194]. Likewise the albumin T1824 bond appeared less stable than the $I^{131}$ bond[56] (Fig. 1).

Since the rate of degradation of the protein-label bond is assumed to define the rate of turnover of the protein, $I^{131}$ appears to be the most reliable label for this type of investigation. However, Berson[20] has cautioned that overiodination of protein (more than one atom of $I^{131}$ per molecule albumin) may cause alteration in the body handling of the protein and give spurious results.

The initial period of the decay curve corresponds to the body equilibration of labeled protein. Figure 1 illustrates that both T1824 and $I^{131}$ give identical results ($t_{1/2}$ = 0.87 day). In view of this, the intercompartmental equilibration of serum albumin or the rate of transfer of albumin through the capillary membrane may be studied equally well with both these labels.

2) *Reutilization of the Isotope Label.* Reutilization of an isotope label would result in a falsely long degradation rate and therefore slower turnover of protein. This could be the case especially with the more rapidly metabolized proteins, for example, fibrinogen and $\beta_1$-lipoprotein. Experimental evidence indicates that extrinsic labels, such as $I^{131}$, are not reutilized[56,194]. However, labels which form an intrinsic part of the protein structure ($S^{35}$) may be reincorporated from degraded protein into newly synthesized protein[205]. This may well explain the apparently slower degradation rate of albumin $S^{35}$ ($t_{1/2}$ = 23 to 38 days) compared to albumin $I^{131}$ ($t_{1/2}$ = 15 days)[8,64,205].

3) *Inhomogeneity of the Labeled Protein.* The chemical purity of labeled protein must be established for use in turnover studies, since contaminant proteins with different degradation rates would provide misleading information.

4) *Local Variations in Metabolic Rates.* It is likely that within the body certain sites are concerned with rapid protein metabolism, whereas other areas are quiescent. Miller[139] has noted the liver of the rat to have a high index of activity for albumin, fibrinogen and certain globulins. Local variations in the handling of proteins are merged into a single pattern in studies of over-all body turnover, as seen in Fig. 1.

5) *Prolonged or Inefficient Mixing of Labeled Proteins Among Various Body*

° The dye Evans blue, T1824, is bound to and transported exclusively by albumin in the plasma and for this reason has been used extensively as a tracer.

*Compartments.* The true fate of any circulating protein is not accurately determined unless complete equilibration between the different compartments is achieved. A striking example occurs in nephrosis, in which urinary loss of circulating labeled protein may be so rapid that compartmental equilibration may not be achieved during a study.

Attempts to interpret turnover of labeled proteins have been hampered further because the ultimate manner of synthesis and degradation remains unknown[199]. Is a protein molecule synthesized simultaneously or serially *in toto* by a template mechanism which assembles specific amino acids or polymerizes preformed peptides? Or, does a protein molecule (once formed) remain relatively stable for a prolonged time being degraded only partially to permit exchange of amino acids or peptides along its chain length? If the former explanation applies the disappearance of label can be construed to reflect the degradation of protein as a functional entity.

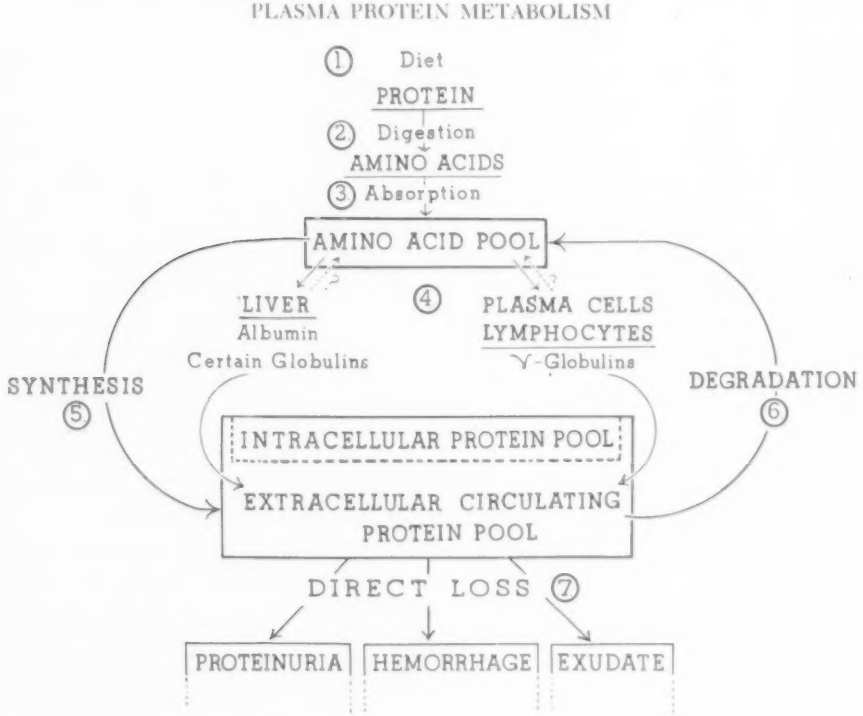


Fig. 2.—The chief factors in turnover of proteins are: The availability of dietary amino acids *1.*, *2.*, *3.*, for synthesis, and equilibration of these with the plasma proteins through the process of synthesis *5.* and degradation *7.* Sites of synthesis *4.* of individual proteins are not established in humans nor is it understood whether degradation of these proteins occurs at same site. Direct loss *7.* of whole protein may induce profound changes in the balance of these pathways.

It is apparent that only with the most exacting specifications in regard to the identity, homogeneity, and measurements can the handling of proteins by

the body be assessed. While the present techniques of determining protein turnover may have certain shortcomings, data from different laboratories have provided at least a fair reflection of changes in protein metabolism induced by different diseases.

**Turnover of Circulating Proteins.** The problems associated with altered growth and development, especially in the early age period, may suggest the possibility of faulty nutrition. An imbalance in the supply and demand of specific nutrients, ranging initially from the diet, down through the pathways to the level of cellular metabolism, must be considered carefully in sorting out possible disorders. Figure 2 illustrates a simplified protocol of the metabolic pathways which may be associated with alterations in the circulating proteins. A deficiency in plasma proteins may occur not only with a general or specific lack of amino acids, (Fig. 2), *1.*, *2.*, *3*, or increased loss of proteins *7.*, but also because of defects in specific turnover mechanisms *4.*, *5.*, *6*. The former aberrations may be reflected by a decrease in total plasma protein concentration; however, involvement of the latter pathways *4.* may induce alterations of a more specific nature requiring special tests for evaluation (for example, agammaglobulinemia, afibrinogenemia, and Wilson's disease).

Evidence substantiating the concept of a dynamic protein turnover process (synthesis and degradation) has accumulated largely since Schoenheimer's tracer studies[177]. Folin's theory[63] of slow endogeneous wear and tear, which existed within a system apart from the handling of ingested proteins, has been seriously questioned. The findings indicate that ingested amino acids mingle with those derived from endogenous metabolism and combine to form a common amino acid pool in which the original source of the amino acid is indistinguishable. In contrast, Cohn and Monod in studies with an *E. coli* system[38,39], have submitted evidence that intracellular proteins are not involved in rapid turnover with extracellular amino acids. In view of these disparate findings, Tarver[199] has suggested that the concepts of "dynamic turnover" of Schoenheimer and "no turnover" of Monod should be taken into consideration in any over-all hypothesis of protein metabolism.

It is beyond the scope of this paper to discuss all of these areas; instead, attention will be devoted to a few of the problems of clinical interest.

ALBUMIN. Since albumin constitutes 55 to 60% of the total plasma proteins, its concentration is used frequently as a reflection of body protein balance. As the major plasma protein constituent, it is concerned with fluid balance, anionic metabolite transportation and acts also as a reserve of available nitrogen. The balance struck (Fig. 2) between albumin synthesis and degradation with or without the complication of direct protein loss, will determine the circulating albumin concentration.

The low values of plasma albumin normally occurring in prematures and newborns were reviewed by Bongiovanni in this journal[26]. Starting close to "edema level" at 3.6 gm.%, albumin increases in concentration reaching adult levels about the age of 5 years. No reports have been found regarding turnover rates in the hypoalbuminemic neonatal period, however, Smith[189] has discussed related factors.

Approximately 4 to 5% of the total body albumin is degraded per day ($t_{1/2}$ = 13.5 days; 10.4 gm./$m^2$/day)[56,194], in the normal adult, and to maintain a balanced state an equivalent amount must be synthesized daily. An increased metabolic degradation of albumin has been demonstrated in thyrotoxicosis by

Dixon[46]. Likewise Sterling[196] has found an increased degradation of albumin $I^{131}$ in postoperative patients. ($t_{\frac{1}{2}} = 6.3$ days; control $t_{\frac{1}{2}} = 12.4$ days.)

Hypoalbuminemia is commonly observed in children because of impaired synthetic mechanisms, as in deficient diet 1 or the malabsorption syndromes 2, and 3. (Fig. 2), (for example, celiac disease, mucoviscidosis, chronic enteritis). Within this area must be considered the hypoalbuminemia of liver disease. Isotopic studies with albumin $I^{131}$ have provided strong evidence that albumin is not degraded faster in patients with cirrhosis than in normal controls ($t_{\frac{1}{2}} = 13.5$ days)[56,195]. Based upon the studies of Madden and Whipple[131] and other biochemical evidence[10], a deficient hepatic synthesis (Fig. 1, 4.) seems the most plausible answer. Miller's[139,140] investigations with perfused rat livers have shown clearly that albumin is synthesized wholly by the liver.

Idiopathic hypoalbuminemia is a rare condition which has been reported by Homburger[98], Schick[175] and others[50,202]. A decreased circulating albumin, despite adequate nutrition and normal liver function, may be present alone or in association with alterations in other plasma proteins. Although diminished albumin synthesis might explain some of these cases, Albright[7], by means of nitrogen balance studies, in one such patient, has reported increased albumin degradation as the mechanism.

The etiology of a transient form of hypoproteinemia with edema in infants and young children likewise remains obscure. In these patients the development of temporary edema associated with a decreased serum albumin concentration occurs in the absence of demonstrable hepatic, renal or nutritional disease[65,216]. Interference in protein synthesis or degradation by infection or allergic phenomena have been postulated.

Loss of protein occurring in proteinuria, hemorrhage, and burns, might be expected to induce a compensatory increased synthesis or decreased degradation of protein. Adams[2] has shown that a normal individual is able to maintain an albumin level in the face of great losses induced by daily plasmapheresis (500 ml. blood, approximately 20 gm. albumin per day for 25 days). Proteinuria may be considered as a modified plasmapheresis; however, studies on the handling of albumin $I^{131}$ in patients with proteinuria have given variable results. Thus Gitlin and Janeway[73] reported an increased degradation rate of albumin $I^{131}$ in nephrotic proteinuria; Eisenmenger[55] calculated a normal degradation rate. In the lesser proteinuria observed in 6 patients with cardiac disease, Bauman[14] reported a decreased degradation rate of albumin $I^{131}$.

Mention should be made that relationships between different plasma proteins may in some manner influence their respective turnover rates. In this regard, Gutman[85] has emphasized that hypergammaglobulinemia is commonly associated with hypoalbuminemia in chronic inflammatory and granulomatous diseases (for example, "collagen diseases," liver diseases, tuberculosis, parasitic infestation). Bjorneboe's observation[22] of the association of hypergammaglobulinemia with hypoalbuminemia in rabbits led to his theory of the control of protein turnover by colloid osmotic pressure.

γ-GLOBULIN. The circulating γ-globulins comprise approximately 11% of the plasma proteins[47]. Only a small fraction of γ-globulins can be accounted for as measurable antibodies[154], and the possibility exists that this family of closely related proteins[186] may have other functions. The wide variations reported

(from 20 mg. per 100 ml. to greater than 7 gm.%; normal = 0.75 gm.%) in diverse pathological conditions have stimulated great interest in the pathways of synthesis and degradation.

Dixon[45] has reported that the turnover rate of γ-globulin $I^{131}$ changes with age. In infants and children the multaneously there is little or no synthesis until approximately the third month of life[142,150].

A profound decrease in synthesis of γ-globulins, initially reported by Bruton[29,30], has been found as a congenital, sex-linked, recessive characteristic occurring in males and described

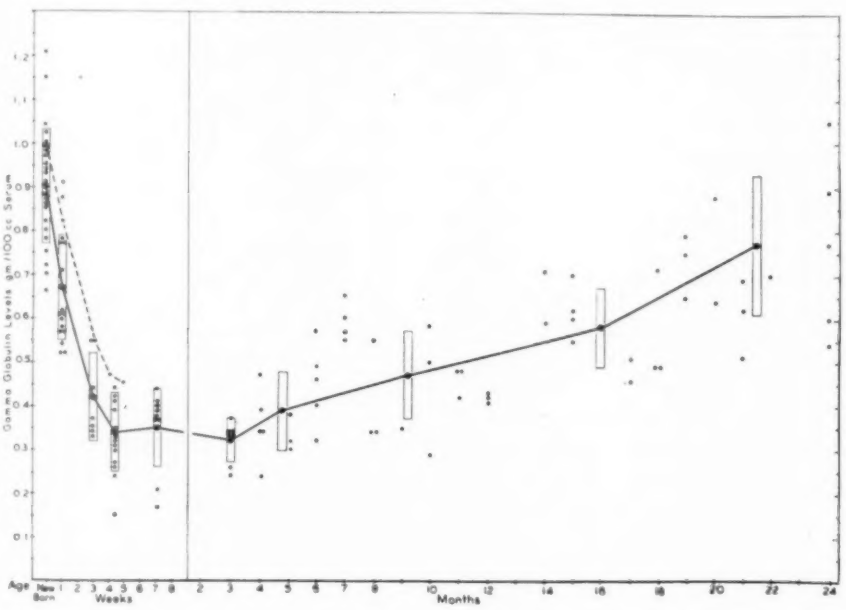


Fig. 3.—Changes in serum gamma globulin levels from birth to 2 years of age, with change in age-scale at 2 months. The dots represent the individual values, the solid line connects the mean values, which are at the mean age of the groups. The bars indicate the range of 1 standard deviation. The dotted line connects the values of a single patient followed from birth to 5½ weeks of age. These alterations in γ-globulin represent the greatest shown by any of the measurable plasma proteins. (Reprinted with the permission of *Pediatrics*.)[150]

half life was longer ($t_{½}$ = 20.3 ± 4.1 days) than in normal adults ($t_{½}$ = 13.1 ± 2.8 days).* Marked changes in turnover occur in the first few months of life. Figure 3 illustrates that the newborn has a full complement of circulating γ-globulins which are degraded in an exponential manner. Si-

as congenital agammaglobulinemia. Numerous accounts of acquired agammaglobulinemia or hypogammaglobulinemia were also reported in older individuals of either sex[171,207]. The association of these phenomena with recurrent and debilitating infections has led to extensive studies into the mech-

*Some question arises regarding the significance of these different rates of degradation of γ-globulin in infants and adults, since the range of γ-globulin $I^{131}$ half-life in normal adults has been found to vary from 10.5 to 21 days in other studies[54,56].

anisms of immunity. Good[78,79,86], has described a deficiency of plasma cells in the hematopoietic tissues and related this to the inability to produce gamma globulin and circulating antibodies (Fig. 2, *4*.). These patients retain normal immune responses to Gram-negative bacterial endotoxins and may develop acute phase serological reactions in a normal manner[78]. Preliminary evidence indicates that direct transformation of parenterally administered nonspecific γ-globulins into specific typhoid antibody does not occur in agammaglobulinemia under the influence of typhoid immunization[172].

Although impairment of γ-globulin synthesis appears to be the primary disturbance, the degradation rate of γ-globulin likewise may be altered. Good[80] reported that γ-globulin is not destroyed more rapidly than normal in these cases. In one boy with congenital agammaglobulinemia, we noted a degradation rate slower than normal ($t_{½}$ = 32 days; normal $t_{½}$ = 15.4 ± 4.0 days). In an adult with acquired agammaglobulinemia Smythe[191] likewise found a slower degradation of γ-globulin $I^{131}$ ($t_{½}$ = 50 days). In passing, it should be noted that agammaglobulinemia can be assessed most accurately by immunochemical methods which are more sensitive than free or paper electrophoresis.

The ability to develop antibodies in the presence of deranged nutritional states and altered circulating γ-globulin levels has been reassessed recently. Balch[9] found no relation between nutritional depletion, total serum protein (albumin or globulins) and the ability of the patient to develop antibodies in the diphtheria anamnestic reaction. In fact the antibody response appeared better in the nutritionally depleted elderly patients than in the normal controls. Similar findings were recorded by Larson[117] in chronically ill patients with elevated γ-globulin levels. An exception was noted by Larson[117] and Zinneman[217] in patients with multiple myeloma. These patients may have a profound decrease in normal γ-globulin production presumably because of a great synthesis of myeloma protein. Very poor antibody production was found despite the great activity of the plasma cells.

The aberrant serum protein patterns occurring in certain patients with multiple myeloma have provided an excellent opportunity for study of a very specific type of hyperglobulinemia. The circulating myeloma protein in patients with multiple myeloma was in most instances immunologically related to, but not identical with normal γ-globulins[125,126,187]. In each patient the myeloma protein had a distinctive immunological characteristic which was individually specific, quite apart from the cross relationship with normal γ-globulin. Bence-Jones protein, which is excreted in the urine in many of these patients, apparently is not related metabolically to the circulating myeloma protein, according to Putnam[160]. The error in plasma cell metabolism which is manifest by these marked alterations in protein turnover remains obscure.

The imbalance between synthesis and degradation which accounts for the hypergammaglobulinemia of chronic liver disease has been studied most frequently with γ-globulin $I^{131}$ [13,55,57,90,91]. An increased degradation rate was found in these patients ($t_{½}$ = 8.3 ± 3 days; normal $t_{½}$ = 15.4 ± 4 days)[55,57]. In a group of young individuals suffering from an unusual type of coarse nodular cirrhosis with marked hypergammaglobulinemia[19,27,111,180] 5 to 10 and in one instance 20 times the normal amount of γ-globulin $I^{131}$ was degraded. Since the rate of γ-globulin degradation rose above normal (1.2 gm.

/m²/day) to as high as 25 gm./m²/day, the maintenance of an increased γ-globulin level in the plasma indicates that a marked increase in γ-globulin synthesis must occur.

In view of these wide changes in γ-globulin turnover and the associated modifications in the immune response, it is of practical importance to know about the manner by which the turnover of this protein is regulated. The successful heterologous skin transplantations done by Good[78,79] in patients with agammaglobulinemia emphasize the fascinating implications of a better understanding of these natural processes, a subject reviewed recently by Burnet[31].

TRANSFERRIN: IRON BINDING GLOBULIN. The alterations in turnover of circulating transferrin bear upon its function of making iron available for hemoglobulin synthesis. Josephs[103] and Granick[82] have reviewed the processes of iron metabolism and little is known about the controls regulating the plasma iron transport system and changes in disease states.

In a healthy adult, plasma transferrin circulates at a concentration of approximately 250 mg. per 100 ml. The values of total plasma iron binding capacity (T.I.B.C.) may be used to reflect the levels of available transferrin. A rise from a T.I.B.C. of 260 μg per 100 ml. at birth through to high values of 420 μg at about 2 years of age, thereafter falling to adult levels of 300 to 350 μg have been recorded by Smith[190].

The circulating transferrin level will be not only a function of the requirement for metallic iron but will depend also upon the state of protein metabolism in the body. Thus the decrease of transferrin in hemolytic anemias (T.I.B.C.) = 250 μg per 100 ml. saturated with the excessive available iron) compared to the decrease of transferrin in chronic infections (T.I.B.C. = 250 μg per 100 ml. but only 40% saturated with iron) may invoke different systems of control for synthesis and degradation of transferrin.

Large losses of protein from the body (Fig. 2, 7.) may vitally affect the plasma transferrin concentration because of its relatively small molecular size (M.W. = 95,000). In the gross proteinuria of nephrosis Lawson and Shulman[118] found low values for the plasma iron binding capacity and suggested renal clearance rates for transferrin approximating that of plasma albumin. Substantiating this concept, fractionation studies of urinary proteins in nephrosis by means of electrophoresis have indicated a prominent β-globulin component[184]. Analyses of this component separated by preparative zone electrophoresis in a starch supporting medium have revealed that it has a sedimentation value of $S_{20} = 5{\cdot}1$ S and a high iron binding capacity[183]. Gitlin[73], using transferrin $I^{131}$ in a study of patients with nephrosis, reported not only great urinary loss but also an increased degradation rate within the body, a finding also noted in comparable studies with albumin $I^{131}$ and γ-globulin $I^{131}$.

Although transferrin is only one facet of what Granick[82] has called the evolutionary inventions of the body for iron metabolism, a broader knowledge of transferrin dysfunction would assist in better understanding the nature of the anemias.

CERULOPLASMIN: PLASMA COPPER-PROTEIN. Clinical interest in copper metabolism has been oriented largely to the derangements which occur in the genetically determined recessive condition hepatolenticular degeneration, Wilson's disease[134]. Copper is an indispensable element serving as a prosthetic group for several respiratory enzymes[66]. Compared to the trace amounts in the tissues, the serum concentration is relatively high, (108 ± 9.8 μg per 100

ml.)[16]. Approximately 95% of copper in the serum is firmly bound in the metalloprotein, ceruloplasmin[32].

The recent investigations of Bearn[15,16], and Cartwright[33] have revealed that a marked decrease in the plasma copper level (60 ± 15.4 μg per 100 ml. characterizes Wilson's disease, and is associated with greatly increased rate of urinary copper excretion, (normal = 15 to 80 μg/24 hrs.; Wilson's disease = 230 to 1450 μg/24 hrs.). Sheinberg[174] using specific antiserum to determine ceruloplasmin found a markedly decreased circulating level in association with the low serum copper concentration. Bush[32] using $Cu^{64}$ reported an excessive absorption of copper by the intestine in Wilson's disease. Bearn in similar studies has revealed that the absorbed copper was initially transported by plasma albumin. Under normal circumstances over 70% of $Cu^{64}$ is transferred from albumin to an $\alpha_2$-globulin component, presumably ceruloplasmin, within 12 hours of oral administration. Negligible transfer occurred in patients with Wilson's disease[17,18].

In Wilson's disease a considerable fraction of the excess copper absorbed is deposited in the tissues (brain, liver)[76]. The amount deposited in any particular tissue is, in large part, an expression of the capacity of that tissue to bind copper. The possibility exists that the copper deposition in the kidney may account for the functional impairment of the tubules, resulting in amino aciduria which correlates directly with the excessive copper excretion[16].

As in the case of altered iron metabolism, an integral relationship between circulating ceruloplasmin and copper turnover exists. Studies of specific synthesis and degradation of this protein may be expected to clarify the picture further.

FIBRINOGEN. Fibrinogen is present in a concentration of approximately 0.3 to 0.4 gm.%. Apart from the relationship between circulating fibrinogen concentration and the blood clotting mechanism, the importance of altered fibrinogen turnover has been emphasized in chronic inflammatory diseases and associated with certain sensitivity states.

Sacks[170] has called attention to the presence of acquired fibrinogen deficiency in "a widely disparate group of hemorrhagic disorders" and possibly associated with abnormal bleeding in some cases of *abruptio placentae*, amniotic fluid embolism, retention of a dead fetus *in utero*, carcinoma of the prostate with metastases and pulmonary surgery. The underlying error of fibrinogen metabolism in this group is not clear.

The less common group of patients with congenital afibrinogenemia has been observed by Gitlin[72] to catabolize injected fibrinogen at a rate with a half life of four days. Since this is close to the normal turnover rate reported by Madden ($t_{½}$ = 5.6 days)[132], a defect in fibrinogen synthesis appears to be the primary disorder in congenital afibrinogenemia. Miller[139,140] has reported that the perfused rat liver synthesizes all of the fibrinogen; consequently congenital afibrinogenemia may represent an isolated hepatic dysfunction.

The concentration of circulating fibrinogen has been reported by Fletcher[62] and others to reflect closely the clinical activity of rheumatoid arthritis. Emphasizing the known dependence of sedimentation rate upon circulating fibrinogen concentration, Fletcher's data ranged from levels of fibrinogen as low as 200 mg. per 100 ml., E.S.R. = 3 to 4 mm./hr. to levels of fibrinogen = 800 to 1,000 mg. per 100 ml. and E.S.R. = 120 to 130 mm./hr. In view of these fluctuations the turnover of fibrinogen must be considerably altered in states of chronic in-

flammatory disease but specific studies of synthesis and degradation remain to be done.

Evidence of the integral role of fibrinogen in the pathological changes associated with sensitivity reactions have been reported by Thomas[201]. Rabbits developing the Shwartzman reaction reveal a disappearance of fibrinogen from the circulation associated with the appearance of intravascular fibrinoid deposits. A qualitative alteration of the fibrinogen appears to occur, converting it to a readily precipitable complex under the influence of heparin. Observations in children succumbing to sensitivity reactions have been reported by Gitlin[75]. Using fluorescent fibrinogen antiserum, the fibrinoid deposits in the affected vessels were identified and appeared to consist in large part of fibrinogen. Of related interest is a report from the same laboratory that the alveolar membrane in pulmonary hyaline membrane disease of newborns contains fibrinogen[74].

LIPOPROTEINS. The lipoproteins are a class of conjugate proteins, the function of which is believed to be the transportation of plasma lipids in a water-soluble complex[153]. Cholesterol, phospholipids, fatty acids and trace amounts of carotinoids and estrogens are circulated in the blood bound to lipoprotein.

Studies of turnover and metabolism of lipoproteins are dependent upon analytical techniques which will provide a clear-cut classification, and a variety of methods have been employed for this purpose[3,89,92,99,144,168,197]. The plasma lipoproteins have been divided into two main groups, the $\alpha_1$- and $\beta$ lipoproteins, the latter representing the class of low density moieties. Certain advantages may accrue from characterization of lipids by means of preparative zone electrophoresis, since the finding of an $\alpha_2$-lipoprotein component in addition to the above by Kunkel[114] emphasizes the subtle distinctions of mobility which this procedure makes possible. Immunological analyses carried out by means of antiserum to highly purified lipoproteins have failed to differentiate between different classes of low density lipoproteins[124].

The manner by which the different lipoprotein components transport cholesterol and phospholipid has been found to vary with age and in different diseases[122,123,153,166,197]. A description of these biochemical variations is beyond the scope of this review. Despite extensive studies of lipid metabolism, there is little recorded about the synthesis and degradation of the lipoproteins. In healthy adults Volweiler's direct studies[205] using $S^{35}$ labeled plasma infusions have shown that $\beta_1$-lipoprotein turns over rapidly ($t_{1/2}$ = 3.5 days, in contrast to albumin $S^{35}$ $t_{1/2}$ = 21 days).

One of the central problems relating to lipoprotein turnover is the manner by which hyperlipemia develops. Increase in the level of total plasma lipids[147] occurs in such diverse conditions as nephrosis, myxedema, diabetes and essential hyperlipemia[6,133] as well as biliary cirrhosis[4,5] and idiopathic xanthomatosis[123]. Since the cholesterol and phospholipids are bound to lipoprotein, it is possible that the protein moiety of the lipoprotein complex will be present also in increased concentration.

Attempts to define alterations in the synthesis and degradation of the lipids in nephrosis were made by London[127] by means of deuterium labeled cholesterol, and a depressed rate of removal of cholesterol from plasma was found (normal $t_{1/2}$ = 8 days; nephrosis $t_{1/2}$ = 45 days). Whether this reveals the behavior of lipoprotein under the same conditions is questionable since a rapid

equilibration of cholesterol between lipoprotein and red cells[87] is known to occur.

Efforts directed to understand the cause of hyperlipemia in nephrosis have so far failed to provide a definitive answer. Thomas[200] has shown that a rough correlation exists between the degree of hyperlipemia and hypoalbuminemia. This explanation, however, cannot apply to the other disease states mentioned.

Experimental nephrosis in animals produced by kidney antiserum simulates closely the clinical picture in humans[10]. In rats Hackle and Heymann[86,93] have shown that the presence of renal tissue is essential for the maintenance of hyperlipemia, however subtotal hepatectomy is accompanied by a considerable decrease in lipemia. The presence of hyperlipemia does not retard the disappearance of lipid (trilaurin or coconut oil) from the blood of nephrotic rats[94] nor does increased mobilization from depot fat appear to occur. Heymann's evidence in nephrotic animals suggested decreased lipid degradation as the cause of hyperlipemia[95]. In view of the functions of heparin in "clearing" the lipemic turbidity from serum[92], Rosenman[165] treated nephrotic animals early in the disease with heparin and observed not only the clearing effect but also a decrease in severity of the renal lesions.

Further studies are indicated to determine the turnover of the lipoproteins *per se*, in addition to observing the behavior of the cholesterol and phospholipid bound to lipoprotein.

Detailed considerations of other proteins of clinical interest, plasma thyroglobulin[163a,203a], the mucoproteins[181, 214a], C-reactive proteins[163], Waldenström's macroglobulin[206], cryoglobulins[193] and the lupus factor[88] will not be attempted in this review. Apart from the changes associated with certain diseases, little is known about their turnover or, with the exception of thyroglobulin, their function.

**Equilibrium of Circulating Proteins.** In order to appreciate more fully the problems of disordered protein metabolism, the turnover of specific proteins, as depicted in Fig. 2, must be integrated with their pathways of circulation throughout the body, thereby providing a picture of their availability for function. Beyond the confines of the vascular tree, areas into which plasma proteins may transfer have been arbitrarily divided into compartments. The manner in which proteins are transferred and thereafter handled will be influenced by individual anatomical and functional peculiarities of these compartments. In addition, the local equilibrium will be established by such controlling factors as intravascular-extravascular osmotic and hydrostatic forces as well as by variations in membrane permeability (for example, capillary membrane). Certain of the mathematical considerations pertaining to intercompartmental equilibration mechanisms have been considered by Solomon[192], Wrenshall[215] and others[21].

The importance of extravascular compartments as reservoirs of plasma proteins was emphasized by Janeway's observation[101] of the rapid disappearance of intravenously infused albumin from the blood (50% within one day). This dynamic ebb and flow of proteins into and out of the vascular system has been illustrated in many experiments by administration of labeled proteins and subsequent sampling of body compartments.

A typical investigation is illustrated in Fig. 1. Following infusion of labeled albumin, rapid mixing occurs within the vascular compartment. Thereafter serial sampling reveals continuing dilution of activity for 3 to 5 days. The initially rapid part of the die-away

curve is characteristic of the distribution phase, during which labeled protein is transferred out of the vascular compartment. Simultaneous observation of another compartment, such as ascitic fluid, reveals a rapid build up of activity due to incoming labeled protein from the plasma.

Following the distribution phase, a second phase of metabolic degradation is characterized by a less steep, exponential die-away curve. After compartmental equilibration has been achieved, it persists, shown by the fact that the activity or concentration of labeled protein within different compartments will decrease in parallel fashion. This second phase defines the rate of labeled protein degradation or protein turnover.

VASCULAR-EXTRAVASCULAR EQUILIBRATION. The rate of transfer of any protein out of the vascular compartment will depend not only on the size and shape of the individual molecules but, among other factors, upon capillary permeability. Considered as a sieve, the capillary pore size may vary in different organs, but little definitive information about this is at hand[99]. The mechanisms influencing capillary permeability have been reviewed by Pappenheimer[156]. The greater concentration of protein in inflammatory and neoplastic exudates compared to simple transudates is evidence that local variation in capillary permeability may occur. In animal experiments, Lake[115] and Miles[137] have recently demonstrated the presence of a capillary-damaging factor. The toxic factor studied by Lake seemed to be associated with a lipoprotein component, since a decrease of the activity followed ether extraction and there was absence of activity in ultrafiltrates.

It is generally believed that the type of equilibrium established between intravascular and extravascular protein determines the colloid osmotic pressure gradient. On this basis, increased capillary permeability has been suggested as responsible for a decrease in vascular protein concentration and thereby extravascular fluid accumulation in nephrosis, cirrhosis of the liver, and the like. However, this has not proved to be the answer. Under normal circumstances the rate of transfer of albumin $I^{131}$ from the vascular compartment is approximately 4 to 5%/hr.[56,194], and that for γ-globulin $I^{131}$ slightly less, 3 to 4%/hr.[57]. In patients with cirrhosis, no increase in capillary permeability to albumin $I^{131}$ was found by Sterling[195], Berson[20], Eisenmenger[56] and this applied likewise to γ-globulin $I^{131}$ [57]. In children with nephrosis Conway[40], after intravenous administration of albumin and γ-globulin (as plasma), revealed a normal capillary permeability to these proteins. These findings are in agreement with the studies of Fine and Seligman[60] upon the problem of "lost plasma" in shock, in which no generalized increase in capillary permeability was found.

Thus the integrity of the vascular system in the maintenance of a central pool of available protein appears to be quite stable in the presence of disease. Forces other than increased capillary permeability would appear in most instances to be the underlying fault creating any major imbalance in compartmental distribution of proteins.

INTERSTITIAL COMPARTMENT: EQUILIBRATION OF LYMPH. While the vascular compartment constitutes only 5% of body weight, the interstitial space into which the plasma proteins are believed to equilibrate represents 20 to 25% of the body weight[67]. However, Berson[20] has reported that plasma albumin $I^{131}$ equilibrates into a space 2.5 times the plasma volume. Since the interstitial albumin concentration may vary in different areas it seems improbable that

the extravascular albumin space would necessarily coincide exactly with the interstitial space based upon electrolyte distribution measurements. In both children and adults approximately 40% of the total body albumin, measured as albumin $I^{131}$, is intravascular whereas 60% is extravascular[55]. The smaller molecular weight of albumin (65,000) may be a factor determining this distribution since $\gamma$-globulin $I^{131}$ (molecular weight = 160,000) is 60% intravascular and 40% extravascular[55].

The importance of the interstitial space as a reservoir of protein was illustrated by Gitlin[70] who reported the widespread distribution of albumin, $\gamma$-globulin, lipoprotein, and iron-binding protein by means of Coons' immunohistochemical technique. In the same laboratory the influence of plasmapheresis upon rapid mobilization of the extravascular pool of protein was clearly defined in rabbits using specific antibodies as the labeled protein[71].

Many factors are associated with edema, or increase of the interstitial fluid compartment; thus nutritional, cardiac, hormonal and renal edema may all have different characteristics[198]. Considered as a protein reservoir, the edema fluid may attain considerable proportions because of increase in volume as well as wide variations in the protein concentrations, for example, from levels below 0.6 to 0.8% to as high as 3 to 4%[48]. Since an accumulation of protein is not as available for function during the storage phase in the swollen interstitial compartment, considerable effort to understand the mechanisms influencing these accumulations has been made.

Alterations in the integral role played by lymphatic drainage are responsible for many of these problems. The important influence of the vascular pulse on spread of substances through tissues was reported by McMaster[138] and Cressman[43] and more recently by Webb[210]. The patency of lymph channels is a prerequisite to proper circulation of interstitial fluid and lymph, and some of the historical studies and investigations in this field have been discussed by Perrott[158].

In the cycle traversed during the extravascular circulation of plasma proteins, the function of the lymphatic system is to collect and channel interstitial fluid protein on the return trip to the vascular compartment. The rapid rate of equilibration of proteins into the extravascular space has been indicated above. In dogs, Wasserman[209] found the appearance of labeled protein in the lymph 7 to 10 minutes after intravenous administration. Similarly Abdou and Tarver[1] recorded a rapid equilibration with lymph in rats, maximal concentration of labeled protein being reached in 3 to 4 hours.

Another example of the factors influencing lymph flow in the thoracic duct is provided by the recent reports of Korner and Courtice[108] which emphasized the more rapid equilibrium of proteins between plasma and lymph resultant to anoxia and to massive fluid infusions. The thoracic duct protein concentration reflected directly the plasma protein levels but turnover of the duct proteins was influenced by such factors as food and fluid ingestion[109]. For instance, Simmonds[182] revealed the effect of fat in the diet in provoking prolonged thoracic duct lymph flow.

The availability of protein for function has been stressed in this report. It is apparent that the anatomical and functional integrity of the interstitial-lymph compartment is of great importance, since obstruction or interference may result in marked disturbances in the availability of proteins for function. Some of these problems will be considered in the following sections.

PERITONEAL COMPARTMENT: ASCITIC FLUID EQUILIBRATION. The peritoneal compartment is one of the segments of the extravascular space which, because of certain anatomical features, is prone to become involved in disturbances of fluid balance. Accumulation of ascitic fluid, like edema, may be simplified to a concept of imbalance in input versus output of fluid in the peritoneal cavity. The biophysical factors underlying such imbalance may vary widely. Thus hypoalbuminemia is responsible in large part for the ascites of nephrosis and malnutrition, but to this must be added the factor of portal hypertension in patients with hepatic disease. Furthermore, obstruction of the lymphatic drainage, either as a congenital defect or as an acquired complication of neoplastic disease, may likewise be a factor in causing fluid accumulation.

The proteins of the ascitic fluid were proven by abundant evidence to be in dynamic equilibrium with the plasma proteins[130a]. Reference to Fig. 1 clarifies this relationship by illustrating the rapid uptake into ascites of labeled protein from the plasma. The factors which influence the migration of proteins into and out of the peritoneal compartment, as inferred above, are poorly understood[55]. Giges[68] has reported that the increased "effective portal pressure," that is, the difference between the colloid osmotic pressure of plasma and of ascites, may be influential in fluid accumulation. The marked improvement in such patients following portocaval anastomosis reflects the etiological role played by portal hypertension[24,55,104]. The possibility of increased hepatic lymph flow as a further source of ascitic protein has been suggested by Hyatt[100] and Volwiler[204].

With respect to the problems relating to fluid and protein drainage, the great permeability of the peritoneal outflow tract has been emphasized by Courtice[42] and Morris[143] in studies on rabbits, guinea pigs and rats. It was shown that entities ranging from the size of plasma proteins (average equatorial diameter = 35 to 38 angstrom units) to the size of red blood cells (diameter = 52,000 to 95,000 angstrom units) could pass freely through the diaphragmatic lymphatics into the pleural compartment. Similar observations of the rapid protein transfer from peritoneal to pleural compartments have been made in humans.

Measurements of the turnover of proteins in the ascitic fluid of patients with cirrhosis revealed that in any given period 5 to 10% of the albumin $I^{131}$ which transfers out of the plasma will enter the ascitic fluid[56]. A comparison of albumin exchange in patients with ascites complicating carcinoma was found similar to the above by Tyor[203], Havens[91], and Bauer[13] have shown that a similar picture prevails in the handling of labeled γ-globulin.

One noteworthy feature bearing upon the protein metabolism in patients with ascites is the apparently slower degradation rates of individual proteins (Fig. 1, albumin $I^{131}$ $t_{½}$ = 19 days, normal $t_{½}$ = 13.5 days)[54,55,56]. On the other hand, in patients without ascites a normal degradation rate of albumin is found. The difference in turnover rates can be accounted for by the amount of labeled protein contained within the ascitic fluid (Fig. 1). After appropriate corrections for this "extra" pool of protein, normal albumin degradation values can be calculated[55,56]. On this basis, the ascitic pool appears to be "metabolically inert" in regard to the degradation of protein, but active in terms of dynamic transfer and equilibration with proteins in other compartments.

In view of the above considerations, the clinical control of patients with

ascitic fluid should be oriented to spare protein losses from the body. The wastage of ascitic fluid by paracentesis removes from the body a pool of otherwise salvagable circulating protein which must be made up by body reserves already under considerable stress. The merits of a restricted sodium intake by means of a low salt diet have been emphasized by Faloon[58], and Eisenmenger[52,53], for the long-term control of fluid accumulation in patients and consequent conservation of protein stores.

PLEURAL COMPARTMENT: PLEURAL FLUID EQUILIBRATION. In view of the aforementioned freedom of exchange between the peritoneal and pleural compartments, and the natural course of lymphatic drainage upwards through the hemiazygos channels into the thoracic duct, it is apparent that many disorders of fluid turnover may be reflected in both compartments or either alone.

James[102] has attempted to define more clearly the etiology of different types of pleural effusion by comparing the differences in venous and pleural pressures. Thus higher pressure gradients, 18 to 20 cm. $H_2O$, were noted in the effusion of congestive failure and constrictive pericarditis, than in the effusion of carcinomatosis or tuberculosis, 6.4 to 8.0 cm. $H_2O$. James furthermore classified the protein concentrations of effusion fluids with relation to the underlying disorder. Thus the protein concentration of a neoplastic effusion is approximately 2/3 that of the plasma, the fluid of cardiac failure or constrictive pericarditis is 1/2 that of plasma and the transudate of nephrosis and cirrhosis 1/4 to 1/8 the protein concentration of plasma. In a comparative study of the plasma and effusion fluid proteins by means of electrophoresis Leutscher[120] found that albumin is present in effusions in similar or increased proportion to plasma, while on the contrary, $\beta$-globulin and fibrinogen are less. In agreement with the above report of James, effusion fluids of cardiac failure revealed an increased albumin concentration which aided in the differentiation from certain transudates in which a lesser proportion of albumin occurred.

The etiological factors inducing accumulation of chest fluid are very complex and again may be thought of as the result of imbalance of input versus output. Chylo-thorax[25,59] or chylous ascites[83,97] represents one of the most problematical situations. Occurring alone, together or occasionally in combination with other lymph accumulations, the situation is one of a "metabolically inert" pool of vital nutrients. In view of the dynamic equilibrium of proteins between the vascular compartment and the thoracic duct chyle, it is apparent that a large lymph flow into the peritoneal or pleural pool will soon place the remainder of the body in a state of protein depletion. It was mentioned above that a decrease in the volume of thoracic duct lymph flow, and thereby a decrease in total protein passing from blood to lymph, is affected by a low fat intake in the diet[182]. In combination with this, it would be interesting to observe the effect of a low sodium intake in controlling the accumulation of fluid in the chest or abdomen of these patients.

CEREBROSPINAL FLUID COMPARTMENT: C.S.F. EQUILIBRATION. In comparison to the magnitude of other body protein compartments, the cerebrospinal fluid pool is small and the influence of its local turnover of protein correspondingly negligible in the over-all body picture. Despite the low protein concentration (15 to 40 mg. per 100 ml.), the diagnostic usefulness of quantitative alterations in C.S.F. protein fractions has been very useful. The various floc-

culation tests (for example, Pandy, colloidal gold) reflecting elevated globulins, and more recently precise immunological estimations of C.S.F. γ-globulin[105], have been of value in observing serial changes in neurological diseases, such as neurosyphilis and multiple sclerosis.

With the advent of paper electrophoresis, which permits separations of very small quantities of protein, numerous reports of spinal fluid fractionation have appeared[164,176,208]. The report of Wallenius[208] made a clear point of the fact that the pattern of C.S.F. proteins frequently reflected the electrophoretic pattern of the serum. Thus elevations of the γ-globulin component in both serum and C.S.F. in cirrhosis of the liver and in multiple myeloma provided strong suggestive evidence of communication between the two compartments.

In discussing the turnover of proteins in the meningeal space, Wallenius considered two sources of C.S.F. protein: 1) choroidal plexus filtering a relatively dilute solution of protein and 2) an area in the lumbar sac described by Eichler and Linder[51] filtering a protein solution of relatively higher concentration of spinal fluid compared to ventricular fluid. With regard to C.S.F. reabsorptive mechanisms, a diffuse uptake of intralumbarly injected substances over the entire meningeal system in addition to the Pacchionian granulations is supported by experimental evidence[51,208].

The dynamic equilibrium of protein between plasma and C.S.F. has not been studied extensively. Fishman[61] compared the turnover rate of human albumin $I^{131}$ in the plasma and C.S.F. of the dog and reported a more rapid turnover in the plasma ($t_{1/2}$ = 5 days) than in the C.S.F. ($t_{1/2}$ = 8 days). Furthermore isotopic albumin transferred from the blood to the lymph in 7 to 10 minutes in the dog but none appeared in the C.S.F. until 40 minutes had elapsed. Similar findings in humans were reported by Chow[34]. Albumin $I^{131}$ given intravenously equilibrated with the C.S.F. within 24 hours. However, given intrathecally, the albumin $I^{131}$ required 20 to 24 hours to equilibrate within the meningeal compartment and thereafter a further 24 hours to equilibrate with the blood. In one attempt Havens[91] was unable to demonstrate the transfer of γ-globulin $I^{131}$ to the C.S.F.

Despite the so-called blood-brain barrier, an equilibrium does exist between the vascular compartment and the C.S.F. or meningeal compartment. The influence of disease on this equilibration, and the sources of C.S.F. γ-globulin, disproportionately elevated in certain neurological disorders, represent only two of the many interesting problems awaiting further investigation.

PLACENTAL PERMEABILITY. Grosser[84] described four morphological groups of placentae in different species based upon the number of tissue layers separating the maternal and fetal circulations. Man, in common with certain rodents, has the thinnest barrier consisting of three layers. Studies carried out in animals are difficult, therefore, to relate to humans, since the placental permeability is also known to vary considerably in different species.

Little can be recorded about the circulation of proteins between the mother and the fetus or about the direct influence of the placenta upon protein metabolism. There are, however, data available comparing the relative concentration of plasma proteins in maternal and cord blood, derived by means of electrophoresis by Longsworth[128], Moore[142] and Mack[130]. Specifically the full term fetus had levels of γ-globulin and albumin close to or slightly higher than the mother. On the

contrary, the levels of $\alpha$- and $\beta$-globulin components were lower in the fetus. These observations have been confirmed in our laboratory by a different technique (salt fractionation)[173].

That the human placenta is permeable to some of the maternal plasma proteins is well established by the correlation of maternal $\gamma$-globulins, identified as specific antibodies, in the maternal and fetal plasma. Thus quantitative studies of antibodies, for example, diphtheria antitoxin, Rh agglutinins, done by Neill[148], Barr[11], and Wiener[212], have demonstrated that these entities when present in the mother are found in the fetus in similar or greater concentration. Moreover these antibodies in the newborn decay in a manner similar to passively acquired antibody and normal $\gamma$-globulin[45,150,185]. Evidence in animals substantiating placental $\gamma$-globulin transfer was reported by Cohen[35] and Kellner[106] in which specific antibodies administered to or developed in the mother appeared in the fetus. Preferential handling by the placenta was shown toward homologous $\gamma$-globulin, since a much slower transfer rate was observed with heterologous protein[35]. These data can be taken as further indirect evidence favoring the maternal origin of antibodies and $\gamma$-globulins, in contrast to the likelihood of their being synthesized by the fetus, a point considered in the section on $\gamma$-globulin turnover.

The above findings refer to the situation existing at the time of full term delivery. That a lesser degree of placental permeability for $\gamma$-globulins may exist in earlier fetal life was suggested in the data of Moore[142]. With increasing prematurity $\gamma$-globulin levels of the cord blood, compared to the mother's, were proportionately lower. Similar findings of antibody levels in mothers and premature newborns reported by Osborne and Dancis[151] substantiate the above concept.

The influence of advanced hepatic cirrhosis in the mother upon the fetal plasma picture was reported by one of us[185]. The increased circulating level of $\gamma$-globulin (1.87 gm.%) was reflected in the cord blood (1.90 gm.%). Despite maternal hypoalbuminemia (2.58 gm.%) a normal fetal plasma albumin concentration was found (3.58 gm.%).

The circulation of lipoproteins across the placenta does not appear great. Russ[167] has reported that ethanol fractionation analyses of maternal and cord plasma indicate not only variance in the $\alpha$-$\beta$-lipoprotein ratios but substantiate the higher lipid values in the mother. This was the finding also noted in the patient with cirrhosis mentioned above[185]. Goldwater and Stetten[77] have demonstrated in the rat that fetal lipid is derived from a dual origin, in part from the carbohydrate metabolism of the fetus and in part transferred across the placenta from the mother.

The influence of the placenta upon protein metabolism in the dog has been investigated by Whipple[213]. Despite the very restricted amounts of whole plasma protein transferred in this species, a well-defined placental uptake of labeled amino acids indicated an important role for this organ in protein metabolism. Of related interest was the finding of an increased turnover of protein in the maternal circulation likely reflecting an increased rate of protein degradation.

The influence which interposition of the semi-permeable placenta between the large and small circulations of mother and fetus remains an interesting unknown. The possible role of maternal plasma proteins permeating through the placenta to circulate *in utero* during the early period of growth may have considerable significance

prior to the development of the fetal protein synthetic system.

RENAL PERMEABILITY: DIRECT LOSS OF CIRCULATING PROTEIN. The clinical significance of proteinuria in the assessment of kidney disease requires no elucidation. Little is understood about the manner by which the human kidney handles protein. In normal circumstances the urine of children is reported to contain approximately 50 mg. (range 6 to 180 mg.) of protein per day[211]. Attempts to characterize the constituents of normal urinary protein by means of electrophoresis have been carried out by Rigas[162] and McGarry[136] and relatively less albumin found than in comparable serum patterns. Absolute identification of the electrophoretic components of normal urinary proteins has not been reported.

The wide variation in amount and character of protein voided in different types of renal disease is apparent from the studies of Bradley[28], Leutscher[119] and others[135,179,184]. Gitlin and Janeway[69] have established the fact that urinary albumin in nephrosis is immunologically indistinguishable from plasma albumin. It has already been mentioned that transferrin is a prominent constituent of urinary protein. To this must be added the suggestion that thyroglobulin is likewise lost in the urine[10]. The identification of specific antibodies, for example, antistreptolysin "O" by Rytand[169], and the immunological definition of γ-globulin in the urine[188] indicate that proteins as large as molecular weight 150,000 are being transferred by the kidney from plasma to urine. No evidence of lipoproteins in the nephrotic urinary protein has been found[184], although lipid bodies are described[157]. It is apparent that the term "albuminuria" is a misnomer applied to the mixture of proteins appearing in the urine.

Sequential alterations in the characteristics of proteinuria have been observed during the course of renal disease by Blackman[23], and defined as a relative increase in γ-globulin excretion with progression toward chronicity of disease. A recent report by Heymann[96] dealing with salt fractionation of urinary protein to observe changes in the albumin/globulin ratio substantiates the above and this approach has been used as a prognostic index in children with renal disease.

The loss of 10 to 30 or more grams of protein per day from patients with nephrosis represents a severe drain of material from the vascular compartment. This, in turn, stimulates mobilization of proteins from the extravascular compartments and eventually precipitates the patient into a state of profound protein deficiency. The alterations in glomerular or tubular function responsible for proteinuria are not understood, despite extensive studies in animals[149,161,178]. Renal permeability appears limited to plasma proteins of smaller molecular weight (up to the size of γ-globulin), and in terms of body metabolism, these have an exceedingly rapid turnover. As mentioned above, it is not yet clear how great may be the compensatory forces of the body for increased synthesis and attempted restitution of these lost proteins.

RECAPITULATION. Many of the subtle relationships between health and disease are determined by the manner in which individual circulating proteins proceed through their life cycle. Nutritional depletion is associated with impaired protein metabolism whether caused by altered synthesis and degradation or by direct loss as in paracentesis or increased renal permeability. The preservation of the total "mobilizable" body pool of circulating protein, even under conditions of altered distribution, will augur for a more rapid and favorable recovery from illness.

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# GYNECOLOGY AND OBSTETRICS

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## HUMAN INFERTILITY

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COMMENTS ON THE PRESENT STATUS OF ITS INVESTIGATION AND TREATMENT. It has been estimated from population studies by demographers and gynecologists alike that 10% of the married population of this country is involuntarily sterile[26,27,30].

Within the first 3 months of marriage, if contraceptives are not used, or within the first 3 months following the cessation of the use of contraceptives, over 60% of American married couples produce a pregnancy. The curve of incidence of conception falls off rapidly thereafter, until, at the end of one year, there is a 10% residue of couples who have not conceived. This is the group generally designated as infertile and on whom it is advisable to institute techniques of investigation and therapy, should they apply for medical consultation. The above-mentioned curve, however, in several studies falls off so rapidly after the first 3 months that it may be legitimate and advisable to institute infertility studies, should the couple so desire, at the end of 6 months, rather than at the end of a year of failure to achieve conception. In the past the thought has been expressed that so many couples conceive anyway between the sixth and twelfth month of attempted pregnancy that as a whole this group should not be considered as infertility problems. A certain amount of doubt has been cast on this concept, however.

Although the recognition of the infertile couple as a clinical entity in both the medical and the lay mind has become in recent years well crystallized, the over-all improvement in results of investigation and treatment during the past 30 years has been singularly disappointing. The problem of infertility as a relatively unexplored and very fertile area for research has been recognized only recently by the gynecologist, urologist and endocrinologist, as well as investigators in the preclinical disciplines. Societies for the study and exchange of ideas concerning infertility have sprung up like mushrooms throughout the United States, South America and Europe; journals devoted to this subject as well as other specialty

journals have published the results of an extraordinarily large number of investigations over the past 30 years. In spite of this interest and effort, however, the over-all success in the treatment of infertility problems remains about what it was 30 years ago when such men as Carey, Macomber, Meaker, Moench, and others postulated the concept of infertility as a clinical entity. Then, as now, somewhere between 30 and 40% of infertile couples produced pregnancies following investigation and treatment.

The investigation of the female, aside from the so necessary general nonspecific history, physical examination and laboratory work-up, still rests essentially on tests for tubal patency, tests of the condition of the cervix, and tests to determine the hormonal and egg-producing potentialities of the pituitary-ovarian interrelationship. Although numerous variations have been devised in recent years, the basic principles of tests for tubal patency were published as long ago as 1920 by Rubin[22]. A test to examine the cervix as a possible factor in infertility was described by Marion Sims as long ago as 1866[25], and very little improvement over Sims' technique has been presented since then. The study of the hormonal and ovulation potentialities of the ovary are of a later vintage. Burch and Phelps[2] having described a technique of obtaining endometrial tissue by biopsy as recently as 1932. The interpretation of the endometrium as a mirror of pituitary-ovarian function has been an object of many studies since then, and the characteristic changes which the endometrium undergoes throughout the process of a normal ovarian cycle have been described by many individuals but possibly most satisfactorily by Noyes, Hertig and Rock in 1950[20]. Another physiological indicator of ovarian hormonal activity is the interesting shift in temperature which occurs during the menstrual cycle. Although described as long as a hundred years ago by von Fricker[28], this phenomenon was reinvestigated in the light of more recent understanding of the hormonal implications of ovulation by Rubinstein in 1938[23]. Since then, hundreds, if not thousands, of studies have been made in attempts to understand the hormonal implications of this mid-cycle thermal rise, with the general conclusions that it is produced by progesterone[5] and that this thermal shift is roughly coincidental chronologically with ovulation[6,10]. This technique, as well as occasional attempts to measure excretion products of ovarian activity[9,14], have been only partially successful in pinpointing the actual time of ovulation. As yet, however, we have no technique for recognizing the production of a viable healthy egg—one that may be fertilized and develop normally[1].

The investigation of these three intrinsic female attributes of fertility has of course been supplemented by many refinements and variations in technique such as the Roentgen-ray salpingogram in determinations of tubal patency and function; chemical, physiological and bacteriological examination of the cervical mucus to assess the value of the cervical canal as a passageway for sperm; and countless different ingenious but frequently unsuccessful methods for the elucidation and identification of pituitary, ovarian, adrenal and endometrial relationships which might affect the patient's hormonal fertility potential.

To all these basic, intrinsic factors must of course be added the realization, as present 30 years ago as it is today, that fertility in both the male and the female is profoundly affected by systemic, glandular or other abnormalities and by environmental and psychologi-

cal factors. No infertility investigation is complete without a thorough and painstaking assessment of the present and past history of the couple, both individually and collectively, of their environment, of their relationship to each other, and of their mutual and individual psychological attitude and basic motivations concerning their problem of infertility.

It is difficult to assess psychological and possible psychiatric problems in connection with infertility, and these factors are being studied more and more thoroughly, but with as yet few finite results except an increasing awareness of their importance[15,16,17]. For instance, the designation of otherwise unexplained amenorrhea as being "hypothalamic" by Albright and Reifenstein[21] bears with it a definite implication of psychiatric etiology. If some unknown humoral mechanism from the hypothalamus (since there are no neural fibers from the hypothalamus to the anterior lobe of the pituitary) stimulates gonadotrophic activity in the pituitary, then we may very reasonably ask what it might be which stimulates the hypothalamus to produce this unknown humoral factor. The association of the limbic system, of which the hypothalamus is a part, to the neocortex has been well described by MacLean[18]. Afferent stimuli from the neocortex to the limbic system occur as the result of (a) spontaneously generated thought processes in the cortex (if there are such things) or (b) cortical activity generated as the result of response to outside stimuli to the cortex through the sensory system. As MacLean says, *this* is the Psyche and by this process of reasoning we have thrust the physiological chain of events involved in hormonal activity back to a psychic trigger mechanism. A recent and intriguing bit of evidence which may lend some support to this speculation is the finding by Guillemin and Rosenberg[11] that the addition of hypothalamic extract to hypophyseal tissue culture resulted in the secretion of a measurable amount of ACTH. That there may be detectable gonadotrophin production in the same experimental environment has yet to be proved because of the difficulty in accurately measuring small amounts of gonadotrophin production, but it seems reasonable that this may be the case.

So far as progress in the study of male infertility is concerned, the findings of the last 20 years may be considered even more disappointing than studies involving the female. Male infertility is still largely diagnosed on a basis of examination of the semen produced by the male partner, and there are arbitrary standards for the identification of male infertility which must be considered largely matters of conjecture. At the present time the only standards of measurement by which a semen specimen can be assayed are the physical and chemical characteristics of the semen itself plus the concentration, motility and gross morphology of the spermatozoa. When one contemplates the fact that each normal sperm has within its head chromosomal structures which are capable of reproducing all the genetic characteristics of the male parent and his ancestors, it is obvious how very inaccurate and crude our present techniques of measuring seminal genetic potency must be.

Until recently it was thought that a minimal number of 60,000,000 sperm per ml. of semen was a requisite concentration for reproduction but, in his study of 1,000 normally productive males, MacLeod[19] has shown that this number may be reduced to 20,000,000, and that men whose counts are consistently lower than this may also be fertile, although their fertility potential is certainly not as great as that of men

with higher counts. Although it must seem obvious that motility of sperm has something to do with their fertility potential, the amount of motility necessary for reproduction can be determined only in a most general way, and this is also true of the morphology of sperm as examined on stained slides. Presumably an abnormally shaped sperm head represents a definitely abnormal sperm, but here again this is purely conjectural and we have no means of knowing whether it is possible for this sperm in the first place to fertilize an egg, and in the second place produce a possibly normal conceptus.

The very complex nature of seminal fluid also indicates that our present crude methods of measuring its degree or lack of normality must give only the vaguest indication of its true characteristics.

The postcoital test as a means of determining the capability of the sperm to get at least as far as the cervical canal is undoubtedly a valuable test in the investigation of both male and female fertility. To date, however, there have been no really accurate correlations of postcoital tests with subsequent pregnancies, so that again there is considerable conjecture as to what is a good and what is a bad postcoital test[3]. It is naturally assumed, if large numbers of very active, normal-appearing sperm are seen in the cervical mucus at a time that is presumed to be the ovulation date, that this represents a good test and that, in the absence of any other infertility factor, pregnancy should occur. If, on the other hand, no sperm is seen postcoitally in the cervical mucus at the ovulation date, this is presumed to be a bad test and represents either failure to produce sperm on the part of the male, or failure to deposit the sperm in the vagina at the time of ejaculation, or some intrinsic female factor which autolyzes or ejects the sperm from the cervical canal. We have no real proof, however, that what is considered to be a "good test" really is a good test so far as its pregnancy producing potentiality is concerned. It may possibly be that what we call a "good test" is really an abnormal condition representing some unknown factor which prevents these sperms from progressing on their way into the tube to possibly fertilize an ovulated egg. The author has seen instances where there were myriads of very active sperm in clear, glairy cervical mucus as long as 5 days postcoitally in infertility patients in whom no demonstrable abnormality could be found. One wonders whether or not this may represent some as yet unidentified abnormality which actually prevents pregnancy rather than enhances the possibility of reproduction.

Therapeutically, various and sundry empirical techniques have been used to try to increase or improve sperm production of males who by our present standards seem to have inadequate spermatogenesis. The individual with complete azoospermia or consistently very low sperm count with the production of many nonmotile and morphologically abnormal sperm is subjected to a testis biopsy in the hope of identifying some testicular abnormality which may produce this abnormal spermatogenesis[8]. Testicular lesions resulting in inadequate spermatogenesis have been fairly well classified and identified during the past 5 to 10 years, and although this is, of course, the first step in devising therapeutic techniques for improvement, no very satisfactory forms of treatment have as yet been produced for any of the abnormalities described. The individual whose azoospermia is due to a block in the epididymis or vas deferens may be subjected to anastomosis by surgery with

about a 4% chance of subsequent, successful sperm ejaculation.

In the various types of inadequate spermatogenesis many varieties of therapy have been tried with no or little success. High-vitamin, high-protein diets, abstinence from alcohol and tobacco, administration of gonadotrophins and male sex hormone have all been followed by unimpressive clinical results. The administration of androgens surprisingly produced a temporary depression in spermatogenesis rather than an increase but it was thought for a while[12,13] that following temporary depression of spermatogenesis there was a "rebound" phenomenon which resulted in a higher sperm count than that which was found at the pretreatment level. Even this, however, is now considered to be invalid, and the treatment of male infertility remains largely empirical and very unsatisfactory.

Although the intent of this paper is not to describe in detail techniques of investigation and therapy of both male and female infertility, a few generalizations concerning some of the above-mentioned intrinsic tests and the hazards of correct interpretation may be of value.

Certainly one of the best known and conventional assays of infertility in the female is the tubal insufflation, or so-called Rubin test, carried out with carbon dioxide. This test presumably provides a qualitative estimation of the tubal factor in infertility. As anyone who has had extensive experience with this test will agree, it not infrequently produces either false positive or false negative reactions, even insofar as tubal patency is concerned, and certainly tubal patency is only one factor in the responsible part which the tube plays in the process of reproduction. It is therefore necessary, frequently, to carry out a salpingogram with some kind of contrast medium to further explore the morphology of the Fallopian tube. That this also is an occasionally inaccurate technique for identifying tubal patency is indicated by the occurrence of pregnancies in patients whose tubes were thought to have been definitely closed as a result of diagnosis by the above methods[7]. It is becoming more and more apparent that not only is the tube a highway which must remain open to permit the passage of sperms and ova, but also it has a very real physiological function, in fact probably several physiological functions, which are necessary for the successful culmination of fertilization and ovum transfer. The peristaltic action of the tube, the activity of the fimbriated extremity at the time of ovulation[29], the ciliary activity of the tubal mucosa, the possible enzymatic action of the tubal mucosa[24], are all tubal functions which cannot be accurately measured by either the Rubin test or salpingogram, and which may be as vital in the reproductive process as tubal patency itself.

Furthermore, even though one or both of these tests may presumably demonstrate tubal patency, one is still faced with the possibility that disease exists in the pelvis which prevents proper migration of the ovum from the ruptured follicle into the fimbriated extremity of the tube. Even though both tubes be open, the pelvis may be so transformed as a result of previous pathological processes that the ovary may be separated by some barrier of adhesions from the open fimbriated extremity, thus preventing proper ovum migration and fertilization. Occasionally the only way to find out whether or not this is the case is actually to observe the tubes and ovaries, either by laparotomy or culdoscopy. Therefore, any technique we have for the measurement of tubal patency must be evaluated in the light of its inevitable inadequacies,

and the search for a mechanical tubal or tubo-ovarian factor must be pursued by other means.

The inability accurately to interpret the postcoital test has already been commented upon and needs no further discussion except to say that here, too, results must be interpreted with caution and realistic consideration of its inadequacies. Undoubtedly false conclusions concerning the cervical factor may falsely measure the couple's fertility potential, and thus the purpose of the test be defeated.

Brief mention has also been made of the many variables in techniques for the interpretation of hormonal activity in the female, and it is only by experience and critical appraisal that any kind of accurate conclusion can be made, even in a general way, concerning the process of ovulation.

Possibly there are two reasons why progress in the investigation and treatment of infertility has been slow and somewhat disappointing during recent years. The first may be called the problem of "species specificity." Characteristics of the process of reproduction, especially in its hormonal aspects, vary tremendously in different species. Whereas in certain types of medical investigation the results of animal experimentation can be transferred almost directly to the human, this is not the case in most problems of infertility. Most animals and humans react very similarly for instance in the field of immunology, and investigation of surgical problems can be carried out very advantageously in many animals, even though anatomically they are not necessarily exactly similar to the human. Reproductive physiology, however, even in the anthropoids, varies tremendously and it is impossible to transfer results of experimentation in one species to conclusions concerning reproduction in another species. Therefore, research in reproduction frequently has had to be confined within the reasonable restrictions of human experimentation, and conclusions from this type of experimentation must frequently be made by inference[4].

The other reason for disappointing clinical progress in this field has possibly been the frequent lack of controls in clinical research. Here again this may be especially true of studies of hormonal activity but in the evaluation of the therapeutics of infertility, there seems to have been a singularly uncritical approach. This may be partially due to the fact that the experimental animal has of necessity had to be the human, and well controlled series under these circumstances are indeed extremely difficult to carry out.

Certainly the field of human reproduction presents a vast unexplored area awaiting the assault of the inquisitive and imaginative mind. It would appear that research in this field has now reached the point where the clinician must largely give way to the physiologist, the chemist, the endocrinologist, the immunologist, the geneticist, for the sake of obtaining further vital information.

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# RADIOLOGY

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## CHOLECYSTOGRAPHY AND CHOLANGIOGRAPHY: A REVIEW OF PRESENT METHODS OF EXAMINATION

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THE first record of the use of Roentgen rays for the diagnosis of biliary tract disease was that of Buxbaum (1898)[30] who reported his "photography" of gallstones. Two years later, the first American account of the demonstration of calcium-containing gallstones was published by Carl Beck (1900)[13]. In 1924, Graham and Cole[67] introduced an entirely new Roentgen method of examination of the gallbladder, utilizing the intravenous injection of tetrabromphenolphthalein. Although early preparations were given intravenously, subsequent media such as Pheniodol (Priodax) and Telepaque have been used orally. When operative and postoperative cholangiography were developed, visualization of the entire biliary tree became possible. The introduction of Biligrafin (Cholografin) in 1953, permitted this to be achieved by a simple intravenous injection.

Progress in this branch of radiology has been remarkably rapid, and a voluminous literature on the subject has accumulated in recent years. This review, while not exhaustive, attempts to survey the pertinent contributions since 1943. Emphasis has been placed on the various techniques of examination of the biliary tract, rather than the pathological implications.

**Anatomical and Physiological Considerations.** VARIATIONS IN BILIARY ANATOMY. Many occur so commonly that they may be considered normal. The widest variations occur where the hepatic ducts join to form the common hepatic duct. Until very recently, the common hepatic duct was thought to be formed by the junction of two main hepatic ducts. Norman (1951)[134], however, describes a third branch: a right posterior branch with a pronounced dorsal curvature, in addition to the right anterior branch and the left branch

which extend antero-superiorly. The left branch has the largest lumen. Occasionally, the right anterior branch may join the common hepatic duct separately at an unusually low level; it may then inadvertently be damaged at operation. In extreme cases, it may even enter the cystic duct. An unusual congenital anomaly in which a direct communication was observed between the right main bronchus and common hepatic duct has been described in a 5-month-old infant[131].

The common hepatic duct passes usually downward and medially, but sometimes transversely, to unite with the cystic duct forming the common bile duct[77]. In most cases the cystic duct enters the common bile duct on the right side, but in 10% it enters the ventral side, in 2% the dorsal side, and in 8% the left side posterior to the common hepatic duct[180]. The upper part of the common bile duct is imbedded in the hepatoduodenal ligament. It passes posterior to the upper duodenum, descending either around the head of the pancreas posteriorly or traversing it to enter the second part of the duodenum. The papilla of Vater marks its site of entrance into the postero-medial aspect of the duodenum. The common bile duct is amazingly inconstant in its direction, varying from the vertical to the transverse. Sometimes its course is sigmoid, and occasionally it may even cross to the left side of the vertebral column[77]. Complete absence of the common bile duct has been described. In a recent case of Sachs (1952)[152], the cystic duct entered the duodenum directly, while the hepatic ducts entered the corpus of the gallbladder.

*Muscular fibers* are usually very sparse in the cystic, hepatic, and common bile ducts of man. The pancreatic duct (duct of Wirsung), the terminal section of the common bile duct, and the ampulla have four intrinsic muscle groups, however: 1) two longitudinal bundles shorten the duct and erect the papilla; 2) the sphincter choledochus surrounds the common bile duct from the point where it enters the duodenal wall to the origin of the pancreatic duct; 3) the sphincter pancreaticus, present in 30% of adults, surrounds the terminal pancreatic duct; 4) in about 16% of adults, there is a well defined annular sheath surrounding the ampulla, the sphincter of Oddi[98]. The distal end of the ampulla contracts under sympathetic stimulation, the sphincter choledochus and sphincter pancreaticus contract under vagal stimulation[95].

In cholangiographic studies, the lumen of the lower end of the common bile duct on entering the duodenum tapers abruptly into a tortuous and narrow pathway. This narrowing should not be confused with stricture. The pancreatic duct usually joins the lower common bile duct, but opens separately into the duodenum in 29% of cases[91]. Even when the two ducts join, the length of the common ampulla is 3 mm. or more in only 20% of individuals[116]. Occasionally, both ducts open into a true duodenal diverticulum[24].

The *gallbladder* is a blind fibromuscular pouch joined to the common bile duct by the cystic duct. An inconstant sphincter (Lütkens' sphincter) has been described by some authors at the junction of the gallbladder with the cystic duct[66,110], but its presence has not been confirmed by others[91].

Congenital anomalies of the gallbladder range from complete absence to duplication of the organ. Aplasia or hypoplasia of the gallbladder is rare. Rains (1951)[144] reported congenital absence in a 61-year-old woman, discovered at operation. Congenital duplication of the gallbladder is more common. In 75% of the cases, duplication

is complete, with separate excretory ducts and orifices entering the common duct. The two gallbladders may have separate serosal coverings[34], and the second gallbladder may even be imbedded in the left lobe of the liver. Provided they are not too closely approximated, it may be possible to remove one which is diseased, leaving the other intact[125]. A bipartite gallbladder with one cystic duct is even more common than true double gallbladder. This deformity should not be confused with the Phrygian cap deformity in which the fundus is kinked upwards on the corpus, or with an obstructive dilatation of the cystic duct. Septa within the gallbladder may occur, usually in the fundus, and run in an annular or a longitudinal direction. An "hour-glass" gallbladder may be produced either by adhesions or congenital malformations. True diverticula of the gallbladder occur at the lower pole as separate saccular appendages, usually with a constricted neck. These contract along with the gallbladder after a fatty meal if the organ is healthy.

"Cholecystopathia cystica" is a rare malformation in which congenital dilatation of the ducts of Luschka is present. The gallbladder wall contains numerous cysts, many of which communicate with the lumen. Intramural diverticulosis is commonly found at necropsy but less commonly at cholecystography. The "Aschoff-Rokitansky sinuses" in this condition are protrusions of the mucous membrane through the muscularis which extend beneath the serosa. The result is a serrated, double-rim gallbladder outline when visualized at cholecystography.

Congenital anomalies of position are numerous, ranging from an intrahepatic gallbladder to one situated in the true pelvis. A large film of the abdomen should be used in every case and may rarely show a left-sided gallbladder[12,68]. Sinner (1948)[166] described a submucosal cystic duct running from the fundus to the collum of the gallbladder and uniting with the hepatic duct distally within the head of the pancreas. There may be an anomalous insertion of the cystic duct into the forked junction of the hepatic ducts, or into the left hepatic duct. Bullard (1945)[28] reported a subcutaneous abdominal gallbladder with no evidence of a hernial sac.

ROENTGEN PHYSIOLOGY. Newman (1933)[132] has shown that a thin watery bile is continually secreted by the liver, and, as it flows into the bile ducts, mucus is added by glands lining the ducts. The gallbladder concentrates bile and acts as a reservoir. Together with the sphincter of Oddi, it may also protect the liver parenchyma against backflow by compensating for intraductal pressure. When the sphincter of Oddi remains closed, the secretory pressure of the liver cells forces dilute liver bile into the cystic duct and gallbladder. The gallbladder starts to fill when the intracholedochal pressure reaches a height of 50 to 70 mm. of water[163]. By the absorption of water and sodium chloride and the addition of mucin, the bile becomes more concentrated and more viscid in the gallbladder. The specific gravity rises from 1.010 for the hepatic bile to approximately 1.034. Even without a fatty meal, the sphincter of Oddi will relax when the biliary pressure is sufficient.

Evacuation of the gallbladder is due to contraction of the weak intramural muscles, stimulated by the hormone cholecystokinin[168]. This substance is produced by the upper intestinal mucosa when in contact with acid, fat, or products of protein digestion. Section of the vagus splanchnic nerves does not affect evacuation[18]. Gallbladder emptying is normally facilitated by the upright posture and by the inges-

tion of a fat-containing meal. Contraction of the gallbladder may also be stimulated by hydrochloric acid, and individuals with hyperchlorhydria may have rapidly emptying and poorly concentrating gallbladders which may be interpreted as pathological on a Roentgen examination with contrast media. As a general guide, it is believed that a normally functioning gallbladder should empty half its contents in 3 hours, and it is usually seen to be significantly contracted 30 minutes after a fatty meal[44].

Volumetric methods for measuring the contraction of the gallbladder have been described by Wieser (1954)[189], and by Albot *et al.* (1953)[4]. The sphincter of Oddi may act independently or in coordination with duodenal musculature; the vagus inhibits and the sympathetic stimulates sphincteric contraction. Rhythmic contractions of the ampulla of Vater and of the lower segment of the common bile duct have been observed, but true peristalsis apparently occurs only when definite resistance is present[6,111].

*Delayed emptying of the gallbladder* may be due to a variety of causes. Biliary stasis, with accumulation of abnormally thick mucus and bile in the gallbladder, may be the result of a fat-free diet or inability to retain food[26]. A fatty meal the day before a cholecystographic examination avoids this difficulty. Normal pregnancy, constipation[99], hypothyroidism, allergy, and other constitutional disorders are frequently associated with sluggish emptying. Many drugs have a profound effect on biliary function. Morphine and allied compounds cause contraction of the sphincter of Oddi, and may also produce occlusion of the neck of the gallbladder at Lütkens' sphincter[153]. Prostigmine usually produces contraction of the sphincter of Oddi alone, but its effects are inconstant. Sodium hydroxide and sodium bicarbonate produce synchronous relaxation of the gallbladder and closure of the choledochal sphincter. Some opaque media such as Priodax may themselves inhibit contraction of the gallbladder[42]. Although once believed to be a true antispasmodic with inhibiting effects on all sphincters, Demerol (Pethidine) is now known to cause spasm of the sphincter of Oddi comparable to that produced by morphine, and use has been made of this fact to improve visualization of the duct system in intravenous cholangiography[5].

*Relaxation of the sphincter mechanism* is evoked by various nitrites and by atropine. Magnesium sulphate, acetylcholine, eserine, histamine, pituitrin, and epinephrin not only relax the sphincter but simultaneously excite gallbladder contraction; their effects are not constant, however.

*Functional disturbances* of the gallbladder and biliary passages may be classified as dystonia (disordered muscular tonicity), and dyskinesia (impaired movement of biliary contents). Dystonia is supposedly due to disturbed neurogenic reflexes. Ivy (1947)[91] considers that motor dysfunction of the biliary passages may produce symptoms, and that biliary dyskinesia as an entity does exist. A disturbance in the reciprocal innervation of the gallbladder and the sphincter of Oddi, with simultaneous contraction of both, may delay evacuation of the gallbladder and give rise to increased intraductal pressure sufficient to cause symptoms. Some workers consider dyskinesia to be due to mechanical causes[22], while others[101] believe that spasticity of the sphincter of Oddi may be responsible.

Reflux of bile into the pancreatic ducts has been cited as a cause of pancreatitis. Hicken and McAllister (1952)[79] describe cholangiographic findings in

100 patients in whom reflux of opaque material into the pancreatic ducts was observed. Biopsy revealed pancreatitis in only 3 of the 100 cases studied. As Kämmerling (1953)[95] pointed out, it is now known that bile is not an activator of trypsin, and moreover, biliary discoloration of the pancreatic duct is seldom found. It appears that reflux of bile into the duct of Wirsung is not a factor of great importance in pancreatitis.

**Roentgenological Diagnosis.** PLAIN FILMS OF THE ABDOMEN. Every examination of the biliary system should include at least one control or plain film of the right upper quadrant of the abdomen. A preliminary water enema with 1% tannic acid should be used to reduce bowel shadows. On the plain film, the edges of the liver, the right kidney and the psoas muscles should be recognizable. One may detect an enlarged gallbladder, gas or residual barium in the gallbladder or hepatic ducts, calculi, soft tissue calcifications and foreign bodies. Stevenson (1953)[170] states that a definite gallbladder shadow on a plain film is indicative of gallbladder disease. In a series of 14 gallbladder carcinomas, 7 showed abnormal soft tissue shadows. Riedel's lobe of the liver may simulate such a gallbladder shadow, however.

*Gas* in the gallbladder walls and adjacent tissues is pathognomonic of emphysematous cholecystitis. When gas-producing organisms infect the biliary tract, gas first appears in the lumen of the gallbladder, next in the gallbladder wall, then in the pericholecystic tissues and later, if at all, in the ducts[158]. This sequence of events differentiates the condition from other causes of gas in the biliary system. Many cases of pneumocholecystitis are accompanied by elevation of the right dome of the diaphragm, a small pleural effusion and a gas pocket, sometimes with fluid levels, in the right upper quadrant[1]. Differentiation from a subphrenic abscess may be difficult in such cases. The causative organisms, according to a review of 23 cases by Mooney and Matzinger (1952)[123] include *C. welchii, E. coli,* and anaerobic streptococci and staphylococci. They point out that since none of these bacteria is uncommon in the gallbladders routinely submitted for pathological study, some lowering of gallbladder resistance must permit gas infection to develop. Gas outside the lumen in the region of the gallbladder indicates perforation and a pericholecystic abscess[151]. Several weeks are required for absorption of the gas after successful conservative treatment. Emphysematous cholecystitis should be distinguished from the excessively rare lipomatosis of the gallbladder which may cause a dark circumscribed ring around the gallbladder. No gas is present in the lumen or wall, however.

*Air in the biliary system* may be due to erosion of stones into the intestinal tract, or surgical anastomosis between the gastrointestinal tract and the gallbladder or common bile duct. Less commonly, air may enter the biliary tract following perforation of an ulcer, or because of incompetence of the ampulla of Vater[156] subsequent to carcinoma of the pancreas, pancreatitis, recent passage of a common duct stone, or recent surgical procedures. Perforated diverticula may rarely be a cause[69]. Simple incompetence of the sphincter of Oddi apparently permitted air to enter the biliary tract in one case reported[53].

Stellate translucent areas seen in the gallbladder region may be the only visible sign of nonopaque calculi, and are due to gas in fissures within the calculi[31]. Such shadows should not be confused with the appearance of air

trapped in the umbilicus, or soft tissue patterns.

Gall stone ileus represents approximately 2% of all intestinal obstructions in adults, and on a plain film of the abdomen diagnostic signs are: evidence of small bowel obstruction; air or contrast medium in the biliary tree; absence or change in location of a previously noted gallstone, or a gallstone shadow in an unusual site[47].

Opaque shadows in the right hypochondrium may be due to calculi of several types, to calcification in soft tissues or to foreign bodies. Not more than 25% of gallstones contain sufficient calcium to be visible, and only rarely are nonopaque stones sufficiently radiolucent to cast a shadow[80]. In most cases of acute cholecystitis, a calculus is impacted in the cystic duct, and recognition of a single stone medial to a collection of calculi is an aid in diagnosis. Opaque gallstones must be differentiated from hepatic, renal and pancreatic calculi, calcified mesenteric nodes, calcified rib cartilages, intestinal fecaliths, residual barium and opaque foreign bodies[170]. Cholecystography, together with oblique and lateral views should help to differentiate most of these conditions. Afflerbaugh (1955)[2] has reported a case where an intragastric gallstone was seen radiographically. Calcification in the wall of the gallbladder, the so-called "porcelain gallbladder," is the result of chronic inflammation and calcareous incrustation of the gallbladder wall with hydroxylapatite. Cohen (1955)[38] recorded the first case of limy bile (milk of calcium bile) in the common bile duct, visible on plain films of the abdomen. Obstructing calculi are usually found in cases with limy bile. In some cases where gallstones cannot definitely be excluded on a plain film, laminagrams, even without the use of contrast medium, may demonstrate their presence[11]. Fluoroscopic demonstration of abnormal diaphragmatic movement may under special circumstances provide important additional information.

EXAMINATION USING CONTRAST MEDIA. a) *Gas.* The significance of spontaneous gas in the biliary tract has already been mentioned in the preceding section; its intentional usage has been superseded by iodine-containing contrast media.

b) *Barium.* Parts of the biliary tract have been outlined with barium following a barium meal or a barium enema[57], often without air being noted in the biliary tract. In the majority of these cases, spontaneous or surgical fistulae are present. In other cases, however, barium may gain entrance into the biliary tract through an incompetent sphincter of Oddi[69,157]. Seventeen such cases were collected from the literature up to 1943 by Faust and Mudgett[53]. Reflux of barium through the sphincter of Oddi has demonstrated the presence of a living ascaris in the common bile duct of one patient during routine barium meal examination[62].

Barium has also been used indirectly to demonstrate biliary pathology. The neck of the gallbladder and the cystic and common ducts form a tubular ring which almost encircles the superior flexure of the duodenum. When this tubular system is dilated because of obstruction by a stone or tumor within the duct, or by an adjacent extrinsic mass, the duodenal bulb, and the first and second parts of the duodenum may suffer compression which may be clearly demonstrated during barium meal examination. Brown and Harper (1946)[27] state that only in the right lateral decubitus position is this pressure defect adequately shown.

c) *Surgical Methods.* 1) *Transduodenal Cholangiography.* This is a new method of examination of the biliary tract in which that part of the duo-

denum containing the orifice of the common bile duct is isolated by two rubber balloons attached to a catheter with a lead tip. A suitable contrast medium of low viscosity such as Diodone (Diodrast) is injected via the catheter into the isolated segment[63]. The aim is to secure opacification of the duct system by regurgitation, but it has so far proven impossible to do this either in normal subjects or on post-mortem specimens. It appears that this method will only be useful after transduodenal sphincterotomy, after transplantation of the common bile duct, or in the presence of spontaneous internal biliary fistulae.

2) *Percutaneous Transhepatic Cholangiography.* This is another comparatively new method of introducing contrast medium into the biliary system, and was first described in 1952 by Carter and Saypol[32] after accidental aspiration of bile during liver biopsy. Leger *et al.* (1952)[105] used a lumbar puncture needle to duplicate the method in two cases and reported success in both.

The method consists of the insertion of a long needle, approximately 1 mm. in external diameter, through the skin, abdominal wall, and liver substance. The needle is inserted above and to the left of the gallbladder and is directed towards the hilus of the liver. In the technique described by Nurick *et al.* (1953)[135], constant suction is applied to the needle, and if a portal radicle is entered, the needle is irrigated and re-directed. When a bile duct is found, a small test injection of 35% Diodrast is injected before the final injection of 20 ml. is made. Nurick and his associates reported success in 5 cases with obstructive jaundice, and failure in 3 cases without obstructive jaundice. Thus it appears that filling and Roentgen demonstration of the biliary system is unlikely unless chronic obstruction has produced considerable dilatation of the intrahepatic ducts. In one of 5 cases with obstructive jaundice, subsequent intraperitoneal leak of blood and bile occurred with death of the patient. The authors attribute this fatality to omission of preparation with Vitamin K, which has since become routine.

Using a similar technique, Magistocchi and Aguirre (1953)[112] were able to make a definite preoperative diagnosis in 3 cases. The author of this review has made use of a similar procedure in 3 cases of obstructive jaundice (unreported) with good results, but believes that the method is probably too hazardous for general use at the present time.

Where an external biliary fistula is present, cholangiography can be done by inserting a fine catheter and injecting a suitable contrast medium of low viscosity. Oily media are not well suited to such procedures because of their tendency to form globules in the duct system. As has been pointed out by Johnson *et al.* (1949)[93] adequate filling of the smaller branches may only occur after contraction of the gallbladder following a fatty meal.

3) *Peritoneoscopic (laparoscopic) Cholangiography.* This method might almost be considered a refinement of the preceding one, and was first described by Marcelle Royer of Argentina[149]. In the method described by Valenzuela (1952)[183] a special double needle is introduced through the abdominal wall and guided into the gallbladder under peritoneoscopic control. The method is not suitable when the gallbladder has thickened rigid walls, or when many pericholecystic adhesions are present. Peritoneoscopic cholangiography should be preceded by ordinary cholecystography, and in the opinion of Valenzuela, should be followed by operation within 24 hours to

avoid possible delayed complications. Royer *et al.* (1954)[150] describe in a subsequent paper how the method may be used to study biliary tract function, and to provide information on gallbladder tone and evacuation, tone of the cystic duct and the sphincter of Oddi, and intracholedochal pressure.

The technique used by Rosenbaum (1955)[148] makes a point of approaching the gallbladder from above, through the liver and gallbladder bed, and not from the free peritoneal cavity. It is felt that this markedly reduces the risk of a bile peritonitis as the liver rapidly covers and seals the puncture in the gallbladder wall. Rosenbaum believes that with this procedure, a laparotomy can be avoided in many cases.

4) *Operative and Postoperative Cholangiography.* Mirizzi (1932)[120] developed operative cholangiography to locate during operation those gallstones (approximately 10%) which otherwise might be overlooked. Most authors[56,74,81,87,115,165] have found the procedure useful, but others[94] have considered its value limited. It has often been pointed out that its effectiveness depends entirely on the technical care with which the examination is done[96]. Some authors emphasize that false negatives may be obtained with cholangiography, especially when "sand" or small nonobstructing stones are present. Sullens and Sexton (1955)[172] hold that cholangiograms cannot be used to replace common duct exploration when findings suggest the possible presence of common duct stones. They agree, however, that calculi may be missed by even the most careful exploration, and that such exploration should be followed by T-tube cholangiography before the abdomen is closed. Caylor (1954)[35] found the results of immediate cholangiography so successful that its employment had become routine in his practice.

Operative cholangiography may be divided into diagnostic cholangiography, also called "immediate cholangiography," and control cholangiography, or completion cholangiography." The first procedure is done as soon as the abdomen is opened by injecting a contrast medium into the common duct, cystic duct, or even the gallbladder itself. The film is studied prior to further operative procedure to obtain information on the position of the extrahepatic biliary system and any variations which may be present. There is considerable variation in the technique of injection[43,55,65,81]. Direct injection into the gallbladder may be employed when its removal is not anticipated. After gallbladder removal, injection may be made into the cystic duct or into the common bile duct, using a fine needle. Improved filling of the intrahepatic ducts may be obtained by clamping the distal end of the common bile duct before injection, but the risk of over-distention and even parenchymal liver damage is thereby increased.

Gius *et al.* (1954)[64] have found operative cholangiography of value in obstructive jaundice in the newborn. In their method, methylene blue diluted with 35% Diodrast is used to achieve both direct and radiographic visualization of the ducts. The injected dye is of immediate use to the surgeon to show anatomical variations in the duct system, and the radiograph later provides documentary evidence of the situation. Swenson and Fisher (1952)[174] describe a method by which operative cholangiography may be used in cases of mucoviscidosis to demonstrate a patent extrahepatic biliary tree: a polyethylene catheter is tied into the fundus of the gallbladder and after flushing with saline, 10 ml. of 35% Diodrast is instilled. The use of a ureteral catheter inserted into the cystic duct is advocated by some workers[90,142], who point

out that a catheter has many advantages over a needle: the catheter more easily traverses the valves of Heister; depth of penetration can be read from the centimeter markings on the catheter; the extra length of the catheter permits the surgeon to remain further from the field of radiation exposure.

*Control Cholangiography* (Completion cholangiography) is performed after the common duct has been opened. The aim is to check the completeness with which the surgeon has explored the duct system and to demonstrate any stones which may have moved down from the hepatic radicles subsequent to exploration. Control cholangiography may be done by injecting Diodrast through a T-tube in the common duct at the end of the operation, or, if the duodenum or ampulla have been opened, retrograde injection may be made. Hughes and Kernutt[80] feel that although immediate operative cholangiography may be a disappointing routine procedure except in cases of obstructive jaundice, and in re-exploration of the common duct, completion cholangiography is of great value.

Since 1942, the technique of operative *radiomanometric* examination of the biliary tract has been developed to combine cholangiography with the study of biliary duct pressure[3]. An estimate of both morphological changes and the tonus of each segment of the biliary system is thereby provided. The procedure may be carried out during operation and postoperatively; its preoperative use has also been considered. General anesthesia with nitrous oxide, ether, and oxygen does not alter the functional status of the biliary system. However, premedication or complimentary medication and curarization must be avoided. Mallet-Guy (1952)[114] has emphasized that any measurement of pressure produced by injecting a contrast medium into the biliary tract cannot be carried out without some error: methane iodine by-products often cause an artificial hypertension in the ducts; oxypyridine iodine by-products (for example, Diodrast) may increase or decrease biliary pressure; the oily contrast media are too viscid for manometric studies.

One of the main difficulties with operative cholangiography has been the difficulty of controlling respiratory movement and the consequent blurring of detail on the films. Examinations made with portable Roentgen-ray units, and without a Potter-Bucky grid are usually unsatisfactory. High capacity (250 ma or more) machines reduce the exposure time, and the coöperation of the anesthetist may help to reduce the depth and frequency of respiration. Slattery and Saypol (1953)[168] have suggested that the difficulty may be solved by placing the film behind the mobilized bile ducts within the abdominal cavity. The film and ducts then move together on respiration.

*Indications for operative cholangiography* include those for surgical exploration of the duct: history of jaundice, small fibrotic gallbladder, hepatic cirrhosis, small stones in the gallbladder, suspected or palpated common duct stone, enlargement of the head of the pancreas, and thickening or dilatation of the ducts[142].

*Postoperative cholangiography* is performed by the injection of contrast medium into the T-tube, and is best performed 7 to 12 days after operation. Its main value is to provide information concerning the completeness of the primary operation. Hicken and McAllister (1954)[78] found residual calculi in 20% of a series of 550 cases from eleven hospitals. They found the incidence of residual stones ten times as great after cholecystectomies performed by inexperienced surgeons as com-

pared with those performed by specialists. The value of operative and postoperative cholangiograms is therefore greatest when surgical skill and experience are somewhat limited.

Johnston *et al.* (1954)[94] have stated that two important factors may be responsible for stones overlooked or left in the ducts at surgery. Small stones may become imbedded in the crypts or in ulcerated parts of the wall of the common duct close to the ampulla of Vater; these are difficult to dislodge and may be missed as discrete filling defects when radiopaque media are passed through the common bile duct. Small stones can migrate into the hepatic duct and smaller hepatic radicles, and are often inaccessible to a probe; such hepatic stones may only be diagnosed from such indirect signs as absence of filling of one hepatic duct when others nearby are filled. Postoperative cholangiography may be of great value in such cases.

The relation between the biliary tract and the duodenum may be demonstrated by a small amount of barium given by mouth, or through an indwelling duodenal tube. McGowan (1945)[118] makes use of morphine and amyl nitrite to study function of the biliary tract during postoperative cholangiography.

An interesting case was reported by de L'Arbre (1953)[102] in which multiple liver abscesses, together with residual stones were shown after cholecystectomy by postoperative cholangiograms. The procedure may occasionally reveal an unsuspected condition, such as the completely divided common duct in a case reported by Hoerr[81].

Miller *et al.* (1953)[119] describe an ingenious device for securing obturation of the lumen of the duodenum and consequent improved visualization of the biliary tract during postoperative cholangiography. A thin rubber balloon 3½ inches long is fitted over the distal end of an Abbott-Rawson tube and is passed into the second part of the duodenum under fluoroscopy. The balloon is sufficiently inflated (using about 75 ml. of air) to obturate the duodenum without giving rise to pain.

Much discussion has centered about the contrast medium best suited to operative and postoperative cholangiography. Originally, liposoluble media such as Lipiodol, or Iodipin were employed, but these have been discarded because of their tendency to droplet formation and failure to provide homogeneous opacification of the bile ducts. Water-soluble media such as Diodrast, 35 to 70%, and Urokon[35,81] have gained favor. To prevent sphincteric spasm, procaine may be added to the contrast media[48], and spasmogenic agents such as morphine and Demerol should be avoided. In Germany, 30% Joduron has proven satisfactory[107]. Some workers favor an aqueous emulsion of ethyl iodophenylundecylate (Pantopaque)[142]. Jacobson and Heitmann (1954)[92] recommend a viscous, water-soluble contrast medium, 25% Diodrast with 1% methyl cellulose. This solution is apparently nontoxic, produces a homogeneous shadow of optimum density, and being miscible with bile does not form droplets.

*Interpretation* of operative and postoperative cholangiograms may present many pitfalls. Exposures should not be made while the injection is proceding or the movement of the contrast medium may obscure small calculi; a contrast medium which is too dense may have the same effect. Normally there should be filling or partial filling of the biliary duct system and passage of opaque medium into the duodenum. Elevation of the left side of the patient is essential to obtain a profile view of the large ducts and the ampulla of Vater. Abnormally, there is dilatation of the ducts[154], shadows indicative of a for-

eign body, or failure of the medium to enter the duodenum. Shadows suggesting stones may be simulated by air bubbles, blood clots, and mucus attached to the lower end of the T-tube. Repeated flushing of the tube and duct with repeated cholangiograms should show sufficient change to differentiate between a true calculus and artefacts[37]. Air bubbles are differentiated by their change in size and shape on subsequent films. Mucus floccules present irregular and elongated negative shadows, while blood clots appear as long, moulded negative densities. Tumor often appears as an irregularity along the wall of the duct. Incomplete filling of a single branch of the biliary tree is strongly indicative of some obstruction such as a stone blocking the branch[134]. Fading of the shadow of the lower end of the common bile duct with funnel-shaped tapering is a normal finding during cholangiography. A dilated cigar-shaped termination seen as far as the duodenum is usually the result of obstruction at the sphincter[154].

*Over-filling of the ducts* should be avoided for three reasons: the raised pressure may simulate pathological dilatation; small concretions and irregularities may be overshadowed by the opaque medium; actual damage may be done to the liver parenchyma. Regurgitation through the liver and subsequent excretion of contrast medium through the kidneys has been reported in 8 cases in a series of 460 operative Cholangiograms[147]. Obstruction of the common duct was always present. Other reported complications of operative cholangiography include cholangitis, multiple liver abscesses and toxic hepatitis[129]. The danger of regurgitating bacteria or other foreign material into the blood is a further reason for keeping the injection pressure low in cholangiography. The procedure may be capable of separating the post-operative mechanical group with pain due to retained calculi from the functional group. Fóti (1952)[56] claims that it has contributed much to such concepts as hypertonic and hypotonic dyskinesia, vesicular intolerance and the hepatic syndrome of Mirizzi. Cholangiography may also be of use to demonstrate a sinus tract or an abscess cavity.

Routine operative cholangiography is becoming more widely used in hospitals where suitable roentgenographic equipment is available. Inevitably, the question of radiation hazard to those concerned will have to be taken into account. In a series of 500 cholangiograms in which an average of four exposures were made per examination, Lidén and Lindgren (1952)[106] found that the operator's hands received the excessive dose of 5r per exposure, and his body, a surface dose of 10 to 15mr if no protection were used. Corrective measures required all operating personnel to stand at least 3 meters from the operating table and the operator's hands to be out of the direct beam. The anesthetist received 20 to 80mr on the forehead and 25 to 115mr on the left hand, so a lead-rubber shield with a lead equivalent of 1.2 mm. was provided with a lead-glass window for protection. Subsequent measurements showed these precautions provided adequate protection.

The uses of cholangiography have been summarized by Sullens and Sexton[172] as follows: immediate cholangiography should be done in all cases of cholecystectomy; completion cholangiography via the T-tube should be performed before the abdomen is closed; a postoperative cholangiogram should be done before the T-tube is removed. In general, properly taken and interpreted operative cholangiograms are of inestimable value to the surgeon, and may in some acutely ill

patients obviate laborious dissection.

d) *Oral Cholecystography and Cholangiography.* In 1924, Graham and Cole[67] introduced the intravenous use of tetrabromphenolphthalein for the examination of the concentrating gallbladder. In 1940, Dohrn and Diedrich introduced iodoalphionic acid, "Priodax" (Pheniodol, Biliselectan), an oral medium with two iodine atoms in the molecule, and an iodine content of 51.38%. The formula of this compound is β-(4-hydroxy-3, 5-Diiodophenyl)-α-phenylpropionic acid. The standard dose of this medium is 3 gm. Priodax is excreted via the liver, is largely reabsorbed from the intestine and finally excreted by the kidneys. It is partially absorbed by the gallbladder mucosa, the amount being increased in acute cholecystitis. This is probably an important cause of poor visualization of inflamed gallbladders. Copleman (1946)[42] showed that Priodax has a definitely inhibiting effect on gallbladder contraction. With this medium, approximately 50% of stones can be visualized. In 5 to 10% of cases, a normal cholecystogram is obtained when gallbladder disease is present. The toxic reactions with Priodax vary in incidence, but average about 34%[52].

In 1944, another oral cholecystographic medium named "Monophen" came into use. This is also a diiodic compound with an iodine content of approximately 52% and the formula 2-(4-hydroxy-3,5-diiodobenzyl)-cyclohexane carboxylic acid. The dosage is 3 to 4 gm.; the roentgenographic results and the incidence of side reactions are comparable to those seen with Priodax.

In 1949, Telepaque (iodopanoic acid) was synthesized by Lewis and Archer. This material has an iodine content of 66.68% and the formula 3-(3-amino-2, 4, 6-triiodophenyl)2-ethyl propionic acid. Telepaque contains three iodine atoms per molecule, a significant step forward as far as shadow density is concerned. Furthermore, its toxicity in animals is one-third that of Priodax. Excretion of Telepaque occurs mainly through the intestine and not the urinary tract[146]. The medium has become widely used, and the literature contains a wealth of comment on its value compared with other oral media. Opacification of the biliary tract is from 30% to 50% greater than that obtained with other media[141], and with the recommended dose of 3 gm. is often so dense that small nonopaque calculi are obscured. Maximum opacity is reached 12 hours after ingestion and is maintained longer than with other agents[128]. Side reactions average 25%[52,171]. The increased cholecystographic density produced by Telepaque results in visualization of cystic and common ducts on serial films in over 80% of cases[21], and has proven of value in demonstrating Aschoff-Rokitansky sinuses in poorly concentrating gallbladders. No delay of gallbladder evacuation occurs with this medium[36,49,127,164,169]. By reducing the dose to 2 gm., a gallbladder shadow of slightly less density may be obtained, unabsorbed bowel residue is diminished, and side reactions are reduced by 4 to 5%[169,187].

Pseudo-albuminuria is known to occur with Priodax, Monophen and Telepaque[83,160]. This is due to the actual presence of the contast medium in the urine, and is not a nephrotoxic effect. A unique case in which a precipitate of Telepaque, or of some iodic derivative, formed in the gallbladder after a repeat cholecystogram has been reported in a recent paper by Theander (1955)[178].

Preliminary reports on another oral contrast medium, iodophenoxic acid, were published in 1953 by Shapiro[162]. The new agent, Teridax, is similar to Telepaque with an iodine content of approximately 66%, and contains 3

iodine atoms per molecule. The formula is 3(3-hydroxy-2, 4, 6-triiodophenyl)2-ethyl propionic acid. With a dosage of 3 to 6 gm., gallbladder shadows are intermediate in density between those obtained with Priodax and Telepaque[162]. Side reactions are few[82], and unabsorbed radiopaque material in the large bowel is rare with single doses. The cystic and common ducts are shown after a fatty meal with about the same frequency as with Telepaque. Teridax does not inhibit gallbladder contractility, is rapidly absorbed from the intestinal tract, and, unlike Telepaque, is excreted principally by the kidneys. As yet, there are no reports on the incidence of pseudo-albuminuria with this new medium.

Vesipaque is the most recent oral cholecystographic medium reported in the literature[192], and is another complex synthetic compound, containing three iodine atoms per molecule. The iodine content is 68.2%, the highest so far attained. With a dosage of 4.5 gm., maximum gallbladder opacification is obtained in 12 to 14 hours, and the cystic and common ducts are frequently visualized. Toxic effects appear slight, but detailed information is not yet available.

Comparison of oral media available for cholecystography has been made by numerous workers[52,159,185,186]. At the present time Telepaque appears to be the contrast medium of choice, particularly if it is given in doses of 2 gm., the larger dose of 3 gm. being reserved for patients weighing over 150 lbs.

The *reliability of oral cholecystography* has been assessed by several workers in recent years[122,126]. After studying 1,340 cases examined at the Serafimerlasarettet (Stockholm) from 1937 to 1948, Wickbom and Rentzhog (1955)[188] conclude that whenever roentgenograms point to a stone, either directly, or indirectly as a defect in the contrast filled lumen, the evidence is almost completely reliable. Using a proper technique, nonfilling or extremely poor filling are almost always conclusive evidence of disease. Poor filling, but sufficient to indicate or exclude stones, is not necessarily abnormal. With few exceptions, normal cholecystographic findings almost always exclude disease of the gallbladder.

*Cholecystography in infants* has not been attended with the same success as that in adults. Harris and Caffey (1953)[73] carried out 27 examinations with oral media in children under 3 years of age, using Priodax and Telepaque in doses of 0.15 gm. per kilogram of body weight. Successful gallbladder studies were obtained in 15 cases. Satisfactory studies were obtained in 12 infants under 6 months of age. The authors emphasize the importance of taking early serial films between the 4th and 9th hours in young children.

*Poor visualization or nonvisualization* of the gallbladder may be due to a wide diversity of conditions. Apart from lesions of the gallbladder itself, such as cholecystitis, cholelithiasis, tumor, and obstruction to the cystic duct, many extrinsic causes may be responsible: 1) failure to ingest or retain the medium; 2) retention of contrast medium in an eosphageal diverticulum (as occurred with one case examined by the author), or retention due to achalasia; 3) gastric retention, due to pylorospasm or pyloro-duodenal obstruction; 4) gastric ulceration[176] or duodenal ulceration[103]; 5) failure to absorb the medium due to diarrhea, pancreatic or intestinal lesions; 6) extensive liver disease, such as cirrhosis; if the bromsulfalein retention is 30% or more, good visualization should occur[45]; 7) physiological biliary stasis due to prolonged fat-free diet[131], or ingestion of a fatty meal before the examination; 8) unduly rapid emptying of the gallbladder, as

in hyperchlorhydria[163]; 9) effects of drugs *(see section on physiology)*; 10) breast feeding[136]; 11) some constitutional diseases such as exophthalmic goitre, diabetes mellitus, pernicious anemia[25]; 12) pathological lesions in the region of the biliary passages and adjacent tissues; 13) failure of the gallbladder to function after trauma, as part of the systemic response to trauma[86]; 14) previous cholecystectomy.

Many *refinements of technique* have been adopted which increase the value and reliability of cholecystography. The examination should include a film large enough to cover the entire abdomen; this will permit the visualization of an occasional left-sided or pelvic gallbladder[51]. Preparation of the patient should include dietary restriction after the evening meal and no ingestion of fat-containing foods from lunch time of the previous day. A mild cathartic may be used the previous night, or a water enema with 1% tannic acid may be helpful to remove gas and feces before the examination. Persistent intestinal gas can be eliminated with 0.5 ml. pitressin, or 0.1 ml. acetylcholine and 5 i.u. of posterior pituitary lobe extract, given subcutaneously[16]. The value of a preliminary fatty meal before ingestion of the contrast medium has been stressed by many authors[26,41,46,70,117,163,184,188]. Another way to relieve biliary stasis is by the administration of bile acids (Decholin or Ketochol) for several days before cholecystography[17]. A second fatty meal should be routine in all cholecystographic examinations after the filled gallbladder has been examined. Its value for demonstrating calculi not previously seen in the filled gallbladder, and for differentiating calculi and papillomata can hardly be exaggerated[72,124,184]. *Oral cholangiography* with demonstration of the cystic and common ducts may be achieved in approximately 80% of patients by taking serial films at short intervals after ingestion of the second fatty meal[41,71,128,164]. Administration of paregoric followed by inhalation of amyl nitrite[181,182], injection of papaverine[89], and injection of morphine sulphate or prostigmine[153], have been tried, either alone or in combination with a fatty meal, to secure visualization of the ducts. All the drugs mentioned tend to have unpleasant side effects, and if any agent is to be used to cause contraction of the sphincter of Oddi, Demerol (pethidine) is the drug of choice[5].

The "water test" described by Casati (1946)[33] was for some time popular, but with improved contrast media its use has been discontinued. Positioning of the patient is important, and in addition to the usual prone views, some workers recommend the right lateral decubitus position to separate the biliary tract from vertebral shadows[97]. Every examination should include at least one film in the erect position to demonstrate the effect of gravity on floating nonopaque calculi and those that gravitate to the fundus of the gallbladder[23]. In many cases, calculi may be shown by no other method[54,190]. The use of gallbladder compression is of great value, both in the prone and erect positions[8,140]. The fixation of papillomata, as compared with calculi, is well demonstrated by erect compression studies[40]. Tomography is occasionally of considerable value to demonstrate nonopaque gallstones obscured by gas and feces[7], and to delineate the ducts after the gallbladder has contracted. Diagnostic pneumoperitoneum may rarely be justified to determine the position and relations of the gallbladder[109].

e) *Intravenous Cholecystangiography.* In 1953, Langecker, Harwart and Junkmann[100] announced the dis-

covery of a strongly hepatotropic substance, adipic-di(3-carboxy-2, 4, 6-triiodoanilide). The neutral sodium salt of this compound was readily hydrosoluble but was not absorbed to any extent in the gastrointestinal tract. Animal experiments showed the toxicity by intravenous injection to be 1/10th that of Priodax and Telepaque solutions of equivalent strength[138].

From this compound, Messrs. Schering A.G. (Berlin) developed the triiodic intravenous contrast medium Biligrafin later in 1953, with an iodine content of 64.32% and the formula sodium N:N'-adipic-di-(3-amino-2, 4, 6-triiodo)-benzoic acid. The iodine is firmly fixed in the molecule and is not removed in the body. Very high concentrations are attained in the liver bile because Biligrafin is actively excreted by the liver cells, and is also a powerful choleretic. Concentration of the contrast medium in the bile ducts depends on the functional status of the liver. After passing through the liver, Biligrafin is eliminated in the feces without re-absorption and relatively dense shadows are seen in the colon several hours after its administration. Normally only 10% of the medium is excreted via the renal system, but where there is disturbance of liver function this figure rises markedly. In such cases a persistent pyelogram may be obtained, and this has been suggested as a sign of poor liver function[59]. Hornykiewytsch[84] was the first to report clinical results of primary cholangiography on 1000 cases examined with Biligrafin in November, 1953. With the release of the new contrast medium, numerous other workers hastened to publish their findings[15,50,130,133,161,173,175].

Administration of intravenous Biligrafin (marketed in the United States as Cholografin by E. R. Squibb & Sons) requires similar preparation to that usually employed for oral media. The original Cholografin sodium and Biligrafin sodium had a concentration of 20%, and the dose varied between 20 and 80 ml.[9]. During 1954, a 40% compound of an iodolithium salt was introduced in Europe and called "Biligrafin forte"[175]. In the United States, a 52% solution, Cholografin Methylglucamine has been introduced, which in terms of radiopacity per milliliter has twice the concentration of 20% Cholografin sodium. The standard dose for Cholografin Methylglucamine is 20 ml. After warming to body temperature, the medium is injected very slowly, in order to avoid nausea.

Films taken 10 to 15 minutes after the injection show the earliest filling of the hepatic ducts and common bile duct. Filling of the gallbladder by the opaque medium proceeds in a bell-like shape within the lumen of the gallbladder downward to the fundus. Without special technique, optimum gallbladder filling occurs 2 to 3 hours after injection. Films in the left anterior oblique position, taken approximately 20 minutes after injection, demonstrate the duct system. Wet viewing of the early films is of great value, so that the subsequent technique may be adapted to suit the particular patient[10]. Later films taken in the prone position demonstrate the gallbladder well.

Side effects with Cholografin are significantly less than with oral contrast media[191]. Allergic reactions occasionally observed in clinical work should not be regarded as due to iodine sensitivity, since separation of iodine from the Cholografin molecule does not occur during metabolism. Repeat studies with Cholografin up to 6 months after the first injection have so far provided no evidence of any developed sensitivity to the complete molecule[178]. Von Hornykiewytsch (1953)[85] and Theander (1955)[178] found the incidence of

side reactions in adults to vary between 6% and 8%. Minimal albuminuria has been reported in 3 cases after the examination[10], and a hypotensive effect has been noted by Püschel (1953)[143]. In 11 children aged from 12 days to 2 years, Cholografin produced no toxic reactions[177].

Important refinements of technique have been developed in intravenous cholecystography. Hornykiewytsch[85] pointed out that during the filling phase of the common duct the sphincter of Oddi begins to open and some bile escapes into the duodenum. By the time the gallbladder is well filled, the ducts are only faintly demonstrable because of leakage of contrast medium. Ludin and Feine (1954)[108] suggested the use of wooden cones to compress the lower end of the common bile duct. Lange (1954)[101] proposed using two separate injections of Cholografin 2½ to 3 hours apart to show both the gallbladder and the biliary ducts on the same film. With this method films are made only after the second injection. Similar results can be achieved by the combined use of Telepaque and Cholografin[121]. Many workers have used morphine to cause spasm of the sphincter of Oddi in order to improve bile duct delineation[20,65,139,167]. However, morphine has extremely undesirable side effects and causes inconveniently prolonged spasm of the sphincter. Demerol (pethidine), on the other hand, is well tolerated and induces contraction of the sphincter lasting usually about 45 minutes[61]. Aldridge (1955)[5] has described a simple method of using intravenous pethidine to secure simultaneous visualization of the gallbladder and the ducts. A preliminary fatty meal is used to evacuate bile from the gallbladder and after this Cholografin is injected at the same time as 25 to 50 mg. of Demerol. The same needle may be used, but different syringes must be employed, as Cholografin and Demerol are chemically incompatible. Excellent filling of the ducts is usually obtained, and the entire examination may be completed within 90 minutes. As an additional aid to duct visualization, tomography of the gallbladder area has been advocated[11,14,65].

Several workers have suggested that intravenous Cholografin might be used as a test of liver function[58,60,155]. On the other hand, Theander[177] believes that there is no justification for drawing any conclusions from cholangiographic studies alone. Oeser and Frommhold[138] have suggested that the tagging of Biligrafin with radioactive $I^{131}$ may prove useful for assessing liver function.

Causes of nonvisualization of the gallbladder with Cholografin may be divided into those due to general factors and those due to hepatobiliary factors. Since the cycle of intestinal absorption required by oral media is eliminated, almost all the general factors responsible for nonvisualization of the gallbladder no longer apply. However, 80% of Cholografin is bound to plasma proteins as a transport medium, so the possibility of pathological composition, or disturbance of formation of the blood proteins should be considered in nonfilling of the gallbladder[138]. Hepatobiliary causes for nonvisualization of the gallbladder with Cholografin include hepatic insufficiency, obstruction of the cystic duct and gallbladder neck by any cause, chronic cholecystitis with gross contraction of the gallbladder, and kinking of the gallbladder neck or cystic duct due to adhesions. Complete obstructive jaundice is one of the commonest causes of failure of the examination with Cholografin.

*Uses and advantages of intravenous Cholografin* compared with other methods of examination are: 1) The gallbladder and bile ducts are visualized,

irrespective of the ability of the gallbladder to concentrate the medium. Many cases not visualized with oral media will therefore show filling with Cholografin. 2) The dosage is known and is constant. The intravenous route eliminates variable factors such as failure of ingestion or poor intestinal absorption[88]. The medico-legal value of this fact has been pointed out by Oeser and Frommhold (1955)[138]. 3) There is consistent demonstration of the intrahepatic biliary radicles, particularly if a spasmogenic agent such as morphine or pethidine (Demerol) is used. "Clubbing" of the smaller intrahepatic branches is a useful confirmatory sign of chronic biliary obstruction. 4) There is a marked reduction of side effects, and toxicity is extremely low. 5) The medium is used in a physiological manner (unlike retrograde cholangiography) and demonstrates patency of the sphincter of Oddi, even in the absence of the gallbladder, by the presence of opaque medium in the intestine. This is particularly valuable in infants with suspected common bile duct atresia[177]. 6) Comparison of radiomanometry-cholangiograms with intravenous cholangiograms may facilitate diagnosis of organic and functional disorders of the bile ducts[76]. 7) Investigation of the postcholecystectomy syndrome is now possible without surgical cholangiograms. Common bile duct dilatation[154], residual calculi, postoperative duct deformities and "reformed gallbladders" may be demonstrated. In animals, cystic duct stumps as small as 6 mm. will dilate following a cholecystectomy. A common bile duct 7 mm. or more in diameter in humans is considered abnormally dilated. Cholografin consistently demonstrates these dilatations, whereas oral media can do so only if the cystic duct stumps develop concentrating powers. 8) The total time for the examination is only 90 minutes, a much shorter time than that required for oral methods. 9) Cholecystangiograms in children have been excellent[177]. Reichelderfer and Van Hartesveldt (1955)[145] found results with intravenous Cholografin superior to those obtained with oral media in 12 patients under one year of age.

Some *disadvantages* of intravenous Cholografin, as compared with other methods of examination, are: 1) The disadvantages and inconveniences attendant upon all intravenous administrations; 2)the higher basic cost of the examination; 3) stratification of the medium and nonopaque bile occurs on erect cholecystograms unless a fatty meal is given; this may be confused with floating calculi[10]; 4) the gallbladder may be opacified even when it is diseased, so that some authors advocate a follow-up examination by oral methods to determine the ability of the gallbladder to concentrate the medium[85]. However, if serial films show no increase in shadow density and no diminution in gallbladder size, there is presumptive evidence of failure to concentrate; absence of stratification in erect films and of dense rim shadows in prone films may be supporting evidence[5]; 5) regurgitation of medium into the duodenal bulb may occasionally simulate an opacified gallbladder in a cholecystectomized patient[10].

*Indications for intravenous cholecystangiography* have been given by many authors[10,39,50,60,65,75]: 1) in the postcholecystectomy syndrome; 2) in preoperative cholangiography to demonstrate calculi in the common bile duct before cholecystectomy; 3) after unsuccessful or equivocal results with oral cholecystographic media; 4) in infants and children; 5) in emergencies, when speed is a

factor of importance; 6) when a tumor is suspected near the porta hepatis; 7) in cases of recent or subsiding jaundice, where it may help to differentiate between infective hepatitis and common duct stone; 8) in functional biliary disorders where the study of the duct system may be helpful; 9) in patients who cannot tolerate oral media.

*Contra-indications for intravenous cholecyst-angiography* are: 1) in patients with coëxisting severe impairment of renal and liver function; 2) in patients showing sensitivity reactions to the test dose.

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# PHYSIOLOGY

PROCEEDINGS OF

## The Physiological Society of Philadelphia

SESSION OF FEBRUARY 21, 1956

SEMINAR ON HORMONAL ACTION

**Introductory Remarks.** NIELS HAUGAARD, M.D. (Department of Pharmacology, University of Pennsylvania). The theme of tonight's meeting is the action of hormones. Hormones have been recognized for a long time as distinct entities with fairly well defined actions in the body. Despite our quite extensive knowledge of the manifold actions of hormones in the intact animal, there is not a single action of a single hormone of which we can truly say that we know the underlying mechanism. We cannot yet describe the action of a hormone in the same way as we can describe the action of an enzyme. Compared with the phenomenal progress in enzymology, the growth of our understanding of the action of hormones has been disappointingly slow.

Recently, great advances have been made in the chemistry of hormones. The work of Du Vigneaud has established the structures of oxytoxin and the antidiuretic hormone, and Sanger in England has found the way in which the amino acids in insulin are put together. Progress is being made in elucidation of the structures of glucagon, growth hormone, ACTH and other hormones.

Renewed interest has been shown in studying the rate of production of hormones by the endocrine glands and the concentration of hormones in the tissues. The first paper on the program is in this field.

A stumbling block in studying various types of diabetes and the action of insulin in the animals has been the lack of a sensitive and not too laborious quantitative method for determination of insulin. Dr. Vallance-Owen of the Postgraduate Medical School of the University of London has developed such a method based on the action of insulin in increasing glucose uptake by the isolated rat diaphragm. He has successfully adapted this method to studies of several important problems of diabetes and insulin action.

The remaining part of the program will be devoted to the field of the mechanism of action of hormones. We are fortunate in having with us people who will be able to speak to us about the action of three hormones: insulin, thyroid hormone and growth hormone.

When we have heard all of the papers tonight, I hope we will be able to be somewhat less pessimistic than I was in my opening statement and that we may say that although it is still pretty dark, it is getting lighter.

**Plasma-Insulin Activity.** J. VALLANCE-OWEN, M.A., M.D., (CAMB.) (Postgraduate Medical School of London, England). Using the technique of the isolated rat diaphragm previously described (**Vallance-Owen** and **Hurlock**, Lancet, **1**, 68, 1954), insulin activity has been measured in normal subjects, diabetic patients and depancreatized cats.

In normal fasting subjects, the mean

plasma insulin activity was 55 microunits/ml. (range 0 to 115 microunits/ml.). One hour after 50 gm. glucose orally the activity increased, achieving a mean of 400 microunits/ml. (range 105 to 850 microunits/ml.). The activity of insulin added *in vitro* to normal plasma was not diminished.

Plasma-insulin activity was studied with the two broad clinical types of diabetic patients. In the obese group who ordinarily do not require insulin treatment, insulin activity was found. The activity in the fasting state (mean 125 microunits/ml.) was greater than that found in normal fasting subjects. There was usually an increase in plasma-insulin activity 1 hour after 50 gm. glucose orally, but the activity did not exceed that found in normal subjects under similar conditions. When a known amount of insulin was added to the plasma of these patients *in vitro*, its activity was undiminished.

In uncontrolled but nonketotic diabetics who require insulin, no plasma-insulin activity was found. When insulin was added to the plasma of these patients *in vitro*, its activity was apparently inhibited. When the patients in this group were controlled, however, plasma-insulin activity was found and then the activity of added insulin was not diminished.

In normal fasting cats, the mean plasma-insulin activity was 110 microunits/ ml. and the activity of added insulin was not diminished. In depancreatized cats, there was no plasma-insulin activity and, moreover, there was an inhibition of added insulin. In "Houssay" cats, there was no plasma-insulin activity and no inhibition of added insulin.

**The Coexistence of Two Glycolytic Systems in Normal Rat Diaphragm Responsive and Nonresponsive to Insulin.** W. N. SHAW, PH.D., AND W. C. STADIE, M.D. (John Herr Musser Department of Research Medicine, University of Pennsylvania). The aerobic formation of lactate from U-$C^{14}$-glucose by normal rat diaphragm *in vitro* in a phosphate-saline medium, pH 7, has been shown repeatedly to be unaffected by the addition of insulin. In contrast, glycogen synthesis from medium glucose is increased by the addition of insulin *in vitro*. The isolation of the glycolytic intermediates by suitable chromatographic means from the diaphragm has shown that insulin increases the conversion of the medium U-$C^{14}$-glucose to glucose-6-phosphate and glucose-1-phosphate while there is no effect upon the fructose-1,6-diphosphate or pyruvate formed. The addition to the medium of certain nonlabeled intermediates, gluconate, 6-phosphogluconate or glucose-1-phosphate, did not dilute the lactate formed from the isotopic glucose. Other nonlabeled intermediates, pyruvate, glucose-6-phosphate, fructose-1,6-diphosphate and 3-phosphoglycerate, did dilute the isotopic lactate. The possibility that a pathway other than the Embden-Meyerhof type exists under these experimental conditions seems remote. A reisolation of nonlabeled fructose-1,6-diphosphate added to the medium along with isotopic glucose showed that the ester was labeled to about the same extent as the lactate. In the same experiment fructose-1,6-diphosphate from the diaphragm was not labeled. Equilibration of U-$C^{14}$-glucose-6-phosphate in the presence of nonlabeled glucose showed the usual effect upon glycogen which contained no labeled carbon. About 10% of the lactate formed arose from the glucose-6-phosphate with no effect of insulin.

The data suggest that two glycolytic systems coexist in the rat diaphragm. One system is concerned with the production of lactate from glucose and is nonresponsive to insulin. The lactate

synthesizing system has no barrier preventing the exchange of phosphorylated intermediates from the medium with the enzyme system itself. The phosphorylated intermediates of the glycogen synthesizing pathway do not exchange with those of the medium. These data appear to be in agreement with the concept that a transport mechanism is involved in the insulin responsive system.

**The Relationship between Oxidative Phosphorylation and the Physiological Action of the Thyroid Hormone.** M. A. Sprites, M.D. (Department of Pharmacology, Hahnemann Medical School). *In vivo* and *in vitro* evidence presented by a number of groups has favored an uncoupling of phosphorylation and oxidation as the primary locus of action of the thyroid hormone. This work has been done with *hyperthyroid* animals only, and the amount of thyroxin added *in vitro* or even injected *in vivo* has amounted to approximately one hundred times the amount already present in the tissues. The *in vitro* experiments, involving addition of thyroxine or triiodothyronine to liver or kidney mitochondria, never resulted in a rise in oxygen consumption. It was then shown that the lowered P:O ratio obtained could be brought back to a normal level if excess $Mg^{++}$ was added. Furthermore a number of chelating agents were found to lower P:O ratios in similar preparations. Finally Martius found, that by decreasing the amount of thyroxin added to such systems to $10^{-6}$ molar concentration, an increase in phosphorylation occurred as opposed to a decrease at higher concentrations. For these reasons it was decided to retest the "uncoupling" hypothesis by the use of hypothyroid animals. If the hypothesis were valid, then these animals should show a rise in the P:O ratio, unless the normal controls were already at the maximal value obtainable. The experimental data from ten normal and ten hypothyroid white rats indicated a rise in the P:O ratio from 2.7 for the normals to 3.6 for the hypothroid animals. These results thus favor the "uncoupling" hypothesis. Assuming the primary locus of action to be the uncoupling, the possibility of this mechanism explaining changes in oxygen consumption and various synthetic rates occurring in thyroid states was discussed and a schema presented.

**Studies on Effects of Growth Hormone on Fatty Acid Metabolism.** A. Allen,. Ph.D., and S. Weinhouse, Ph.D. (Temple University and The Institute for Cancer Research, Philadelphia). It was observed that the growth hormone did not influence the rate of conversion of C-14 labeled fatty acids to $CO_2$ or acetoacetate in rat liver slices; this was true whether the hormone was added *in vitro* or injected prior to sacrifice of the animals.

Studies *in vivo* demonstrated similarly that injection of the growth hormone did not materially influence fatty acid oxidation by the rat.

Liver slices from rats injected with growth hormone a few hours prior to sacrifice exhibited a somewhat lower incorporation of C-14 labeled acetate into fatty acids and an increased incorporation into cholesterol. These effects, however, were neither marked nor consistent.

High levels of growth hormone *in vitro* produced a generalized inhibition of tissue respiration. This inhibition was not believed to have physiological significance, since it was obtained also with heated and dialyzed preparations of the hormone.

# BOOK REVIEWS AND NOTICES

A Textbook of Medicine. Edited by Russell L. Cecil, M.D., Sc.D., Professor of Clinical Medicine, Emeritus, Cornell University and Robert F. Loeb, M.D., Sc.D., D. Hon. Causa., LL.D., Bard Professor of Medicine, Columbia University. 9th ed. Pp. 1786; 201 ills. Philadelphia: W. B. Saunders Company, 1955. Price, $15.00.

Four years have elapsed since the last edition of this well known textbook of medicine. To bring the volume up-to-date many new sections have been added, including (to mention but a few out of many) Dehydration and Fluid Balance, Acute Nonspecific Pericarditis, The Painful Shoulder, Nonpurulent Meningitis, and others. As has been true in the past, the authors of these sections have been well chosen, and their contributions are well written. A proper emphasis upon the physiologic and biochemical aspects of disease has been maintained, and this has been combined with the disease descriptions rather than being placed in another section of the book. The high standard of previous editions has been thus maintained, and it is certain that this textbook will continue to hold its position amongst the best available.

If there is any complaint the reviewer has to offer it is one he feels should be directed at all texts of medicine. This concerns the problem of emphasis. As illustrated in the present volume, it might be noted that the rare inborn errors of metabolism are given 10 pages while disorders of hydration and acid-base equilibrium are considered worthy of only 13! Psychosomatic Medicine and the Psychoneuroses together fare somewhat better (23 pages), though still entirely out of proportion to their place in the average physician's practice. It is to be hoped that future editions of this and other medical texts will strive to readjust this emphasis. T. D.

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Bone and Joint X-ray Diagnosis. By Max Ritvo, M.D., Assistant Clinical Professor of Radiology, Harvard Medical School. Pp. 752; 568 ills. Philadelphia: Lea & Febiger, 1955. Price, $20.00.

This book was intended primarily for the roentgenologist and those in training in the field of radiology. It has value also for the orthopedist and anyone interested in the study of bones and joints. The opening chapter is devoted to general considerations including discussion of morphogenesis of the skeleton and development of the bones. Tables of appearance and fusion of ossification centers have been included for reference. There is a large chapter on hereditary, congenital and developmental disturbances. (One wonders why the anemias in infancy and childhood were included in this chapter.) Other chapters include Traumatic Lesions; Infections; Glandular, Nutritional and Metabolic Disturbances; Bone Changes due to Chemical Substances and Physical Agents; Diseases of the Joints and the Periarticular Tissues; Bone Tumors; Diseases of the Spinal Canal; and Soft Tissue Roentgenography.

From the critical point of view one must mention the occasional errors (mainly typographical but also some factual) which crop up throughout the text. Also, the space allotted to a subject is not always proportional to its importance. H. W.

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Premature Infants. By Ethel C. Dunham, M.D., Director, Division of Research in Child Development, U. S. Children's Bureau; and Consultant in Pediatrics, World Health Organization. 2nd ed. Pp. 459; 39 ills. New York: Paul B. Hoeber, Inc., 1955. Price, $8.00.

This book has been prepared as a source of information in regard to prematurity and as a guide for the general care of the premature infant.

Part I is devoted to general considerations and to definitions and criteria of prematurity; the incidence, causes and prevention of premature births; and the growth, development and prognosis of premature infants. This section is very complete and is an excellent source of reference.

The clinical considerations of prematurity in Part II are good; consideration is given to the appraisal, care and feeding of well premature infants. The physiologic handicaps of the premature infant are wisely included in the discussion of each system rather than in a separate section. Excessive space is devoted to the chapter on congenital malformations. The section on the pathological states is quite complete. The statement that 95% of cases of erythroblastosis fetalis are due to the Rh factor and only 5% to A or B blood group incompatibilities is not in ac-

cordance with current data. Many of the tables listing the diagnostic features of the various pathological states will be of value to the reader.

Part III is devoted to public health considerations and contains valuable statistical information on the causes of death and the factors influencing the death rates of premature infants. Programs for the care of premature infants on a hospital, local community and state wide basis are also listed.

The appendix contains detailed instructions in the various feeding techniques; illustrations and instructions for the study of neonatal mortality, and an outline of nursery procedures.

The bibliography is good.

This book can be highly recommended to the general practitioner, the pediatrician, the medical student and the nurse who is concerned with the care of the premature infant.

N. K.

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LECTURES ON THE SCIENTIFIC BASIS OF MEDICINE. VOLUME III, 1953–1954. British Postgraduate Medical Federation. University of London. Pp. 398; 56 ills.; 9 plates. London: The Athlone Press, 1955 (John deGraff, Inc., New York, U. S. Agents). Price, $6.00.

THE rapidly increasing number of physicians who are finding this series of volumes of great value to them will welcome the appearance of this, the third of the series. It is to be hoped that many others also will become aware of the tremendous educational value of this presentation of lectures given by outstanding authorities on basic medical topics. Commencing with "Science and History" by H. E. Sigerist, the subjects discussed include Biological Synthesis, The Genetics of Some Biochemical Abnormalities, Tissue Repair, The Supporting System and its Disorders, Hemispherectomy and the Localization of Function, Anticholinesterases, Acetylcholine and the Maintenance of the Cardiac Rhythm, The Growth Hormone of the Anterior Pituitary Gland, Stress and Thyroid Activity, The Physiological Actions of the Sex Hormones, Acid and Alkaline Phosphatase in Disease, Body Water Control, Reactions to Bacterial Invasion, Antiviral Immunity, The Action of Bacterial Enzymes on Immunizing Antigens, Causes of Failure in Antibiotic Therapy, Antimalarial Drugs, Chemotherapy of Cancer, The Scientific Approach to Dermatology, and Experimental Psychopathology.

Although intended primarily for physicians in academic and research medicine, it is the reviewer's opinion that no physician imbued with the desire to keep abreast of advances in medicine will fail to profit from a careful reading of this series each year.

T. D.

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ADVANCES IN PROTEIN CHEMISTRY, VOLUME IX. Edited by M. L. ANSON, Lever Bros. Research Center, Edgewater, New Jersey; KENNETH BAILEY, University of Cambridge, Cambridge, England; and JOHN T. EDSALL, Biological Laboratories, Harvard University, Cambridge, Massachusetts. Pp. 527; illustrated. New York: Academic Press, Inc., 1954. Price, $10.50.

THIS volume continues the contemporary history of protein and their constituent metabolic units, the amino acids. The topics include: 1) The metabolism of glycine. 2) The digestion of protein and other nitrogenous compounds in ruminants. 3) The resolution of racemic $\alpha$-amino acids. 4) Naturally occurring trypsin inhibitors. 5) The formation, composition and properties of the keratins. 6) The molecular structure of simple substances related to proteins. 7) Protein-protein interactions. 8)Physicochemical and biological aspects of proteins at interfaces.

As usual with such volumes, it is impossible to describe the contents of the articles. Their authors are well-known workers in their fields, and located in widely scattered centers; London, Scotland, Japan and the United States of America. The excellent author and subject indices together with the numerous references to the current literature make the volume most useful as a research adjunct.

J. B.

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PÄDIATRISCHER RÖNTGENATLAS. BY DR. M. A. LASSRICH, PROF. DR. R. PRÉVÔT, AND PROF. DR. K. H. SCHÄFER, Hamburg. Pp. 333; 700 ills. Stuttgart: Georg Thieme Verlag, 1955 (Intercontinental Medical Book Corporation, New York, U. S. Agents). Price, $27.40.

As the title of the book expresses, it is supposed to be an atlas and not a textbook. The authors, who are outstanding in Pediatrics as well as in Roentgenology, have collected from several institutes and pediatric clinics composite material over many years. Seven hundred cases were chosen which are especially valuable for teaching purposes. The atlas shows normal and pathological roentgenographic pictures, arranged according to the different organ systems. Every chapter has an introduction in which the indications, the course of examinations, fluoroscoping and

roentgenographic techniques, as well as the different special techniques (planography, bronchography, arteriography, angiocardiography, and others) are discussed.

In the first chapter the normal and pathological findings of the thorax of the child are described. The reproduced pictures are explained by instructive drawings. A series of pictures is to be found, showing the normal neck and lung structure in children in the different age groups. Following is a series of normal and pathological bronchograms. After reproduction of normal lung films a series of the most different pathological lung findings in children is given, covering also the different forms of pneumonia. Another chapter is dedicated to tuberculosis in children, which is illustrated by excellent roentgenograms. Then follows a series of films, covering the pathological changes in the mediastinum and of the diaphragm. In the third chapter normal and pathological findings of the cardiovascular systems are considered. Almost all congenital heart diseases are illustrated by excellent angiocardiograms. Then the reader will find a well chosen series of pictures of the intestinal tract and of the abdominal organs. The congenital and the acquired pathology of the esophagus is covered in all particulars. The gastrointestinal tract is illustrated by superb films of the mucosal patterns. The examinations of the small intestines seem to be extremely important for the pediatricians. Excellent films of nutritional allergy, enteritis, tuberculosis, and others, are shown.

In the chapter about the colon we see good radiographs of malrotation and displacement by extrinsic processes. Hirschsprung's disease is characterized by very good roentgenograms and instructive drawings. The next discussion concerns the congenital and acquired pathology of the urinary system and of the adrenals. The next chapter discusses the development of the skull in the different age groups in babies and in children. The hereditary craniostenoses and endocrine diseases are discussed. Fractures and inflammatory processes are described in an unusually complete way. We also find a description of the intracranial tumors, demonstrated by pneumoencephalograms and arteriograms. Another chapter contains the development of the spine in babies and in early childhood with discussion also of congenital and acquired pathological defects.

Finally in the last chapter the bone pathology in children is described. Summarizing it can be said that this atlas contains a collection of roentgenograms which are excellent in technique. They are chosen on the basis of the great experience of the authors and, therefore the exactly typical pictures in every case are reproduced. This book enables pediatricians as well as roentgenologists to find typical films of pathological changes in an easy way. The format and binding of the book are excellent.

W. B.

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CEREBRAL VASCULAR DISEASES. Transactions of a Conference held under the auspices of the American Heart Association. Edited by IRVING S. WRIGHT, M.D., Chairman, and E. HUGH LUCKEY, M.D. Pp. 167. New York: Grune & Stratton, Inc., 1955. Price, $5.50.

THIS volume places on record the transcript of a conference on cerebral vascular diseases conducted according to the general plan of the Macy Conferences. A group of 34 authorities participated, and the editors have attempted to retain in the transcript the informality and provocative exchanges between these men whenever consistent with the plans for publication. The result has been a very readable and worthwhile volume on a subject of vast practical importance. It is to be hoped that further conferences of this type will be held and similarly reported upon. It would appear that the term "fecundity of aggregation" which has been used to apply to the fruits of the Macy Conferences is entirely applicable to this one as well.

T. D.

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SYMPOSIUM ON ATHEROSCLEROSIS. IRVINE H. PAGE, Chairman. Pp. 249; 54 ills. Washington, D. C.: National Academy of Sciences-National Research Council (Publication No. 338), 1955. Price, $2.00.

THE symposium, of which this volume is a transcription, was held in March, 1954. Its purpose was to gather in one volume current information bearing on the development of diagnostic and predictive criteria and at the same time to bring into clearer focus the status of research at that time and the most promising avenues for future investigation. To aid in integration of the material, each paper was circulated in advance and later revised in the light of the lively and critical discussions that developed. The 26 participants were well chosen and are recognized authorities in the various fields related to atherosclerosis. The anatomy and physiology of the blood vessel wall are discussed, together with its responses to injury and other influences, the role of nutrition, and the metabolism of lipids

and lipoproteins. A section is also devoted to the applications of new techniques such as polarization optics, Roentgen-ray diffraction and absorption, and electron microscopy. Each of the five sections of the book is followed by an excellent summary of the discussion in that particular section, a feature which should prove to be a very popular one. As is true of most symposium transcriptions, there is no index. Although there have been some new developments in the field since the symposium was held, this volume should be of great value to investigators in this field, and to all who are interested in the excellent progress that is being made toward an understanding of our leading public health problem. T. D.

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THE SCIENCE BOOK OF THE HUMAN BODY. BY EDITH E. SPROUL, M.D., Associate Professor of Pathology, Columbia University. Pp. 232; 92 ills., 32 color plates. New York: Franklin Watts, Inc., 1955. Price, $4.95.

THIS book was written for the lay person. The author has attempted the difficult task of condensing and simplifying the vast complexities of human anatomy and physiology into one small volume. Many simple illustrations are used. The volume has value as a basic guide in understanding the human body, provided the material is supplemented by professional or learned clarification. Otherwise, many portions, particularly those dealing with biochemistry, enzymes and physiology may still remain enigmatic for the average person.
W. L.

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THE MANAGEMENT OF ORAL DISEASE. BY JOSEPH L. BERNIER, D.D.S., M.S., F.D.S., R.C.S. (ENG.), Colonel, Dental Corps, United States Army; Chief, Oral Pathology Branch, Armed Forces Institute of Pathology. Pp. 801; 1001 ills. and 5 color plates. St. Louis: C. V. Mosby Co., 1955. Price, $15.00.

ANYONE reviewing this book cannot do otherwise than grade it as an excellent text. The material throughout the book is presented in readily located headings with easy descriptive reading, making for practical guidance to the student or busy practitioner.

Additional color plates to the five shown would have enhanced the illustrations in general. However, the cost of color reproductions would, undoubtedly, have made the purchasing price prohibitive to those who keep abreast of the newer text material.

This book deserves a place in the library of all medical and dental schools, and on the desk of busy dentists and dental specialists.
J. C.

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OBSTETRICS. BY J. P. GREENHILL, M.D., Senior Attending Obstetrician and Gynecologist, Michael Reese Hospital, Chicago. 11th ed. Pp. 1008; 1170 ills., 114 in color. Philadelphia: W. B. Saunders Co., 1955. Price, $14.00.

THE enlarged and profusely illustrated eleventh edition of Greenhill's OBSTETRICS, formerly titled The Principles and Practice of Obstetrics, is as complete and encyclopedic a single volume on the subject as were the former editions originally prepared by DeLee and later by its present author. Many changes have been made both in the arrangement and in the actual content of material, the most notable addition being an extensive consideration of endocrine changes during pregnancy and diseases of the endocrine organs.

Much of the book has been rewritten and although the text for the most part is in agreement with modern concepts there still is a considerable amount of information which might well be re-examined. A minor example of this is his advice that intercourse should be avoided "when the menstrual period would have occurred if the woman were not pregnant. It is at these times that the greatest danger of a miscarriage exists." Another is the section on regional anesthesia. Greenhill has always been antagonistic toward spinal anesthesia for delivery and his frequent warnings as to its danger have undoubtedly eliminated many anesthetic deaths. His long section on saddle block anesthesia however certainly demonstrates the fact that he is unfamiliar with the procedure. For instance he states ". . . Some paresis but no paralysis of the thigh and leg muscles occurs. . . ." "Nausea and Vomiting occur frequently . . ." and "Another annoying postanesthetic complication is urinary retention . . ." (which he infers is frequent).

This text serves a better purpose as a reference for physicians with a considerable amount of obstetric experience than for medical students, interns and general practitioners because it is remarkably comprehensive but often nonspecific. For instance, the dosage and the effects of analgesic agents upon mother and child are described at length but at no time does the author indicate how and when they should be used nor does he outline his own method for administering them. The

same is true of his consideration of the treatment of pregnancy toxemia; the results of the experiences of others with a multitude of preparations and procedures are presented without evaluation and without recommendation as to which are satisfactory for general use.

This book should be outstanding because its author is experienced and widely read, it is well illustrated, the references are well chosen and the text is easy to read but unfortunately it falls far short of its potentialities. It could be considerably improved by reducing or even deleting much of the material describing the reports of unproven methods and drugs which may actually confuse rather than enlighten the problem under discussion; this should shorten the text a good bit. The author should then outline specifically his experience and his recommendations for managing the normal pregnant woman and the complications which may develop. J. W.

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## NEW BOOKS

*The Thyroid Gland.* Edited by P. Eckstein and S. Zuckerman. Memoirs of the Society for Endocrinology, No. 1, Proceedings of a Conference by the Society for Endocrinology at the Medical Society of London, February, 1953. Pp. 48; 19 ills. London: Dennis Dobson, Ltd., 1953 (Cambridge University Press, New York, U. S. Agents). Price, $2.00.

In this collection of papers presented at the meeting of the Society for Endocrinology and devoted to the topic of the thyroid gland, most of the reports deal with investigations into various aspects of thyroid physiology and have already appeared in the literature. Since the field of thyroid physiology and pathology is a vast one, and the reports presented here are not particularly related to each other, one questions the value of publishing them under one cover. B. C.

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*The Determination of Adrenocortical Steroids and Their Metabolites.* Edited by P. Eckstein and S. Zuckerman. Memoirs of the Society for Endocrinology, No. 2, Proceedings of a Conference by the Society for Endocrinology at the Medical Society of London in May, 1953. Pp. 90; 36 ills. London: Dennis Dobson, Ltd., 1953. (Cambridge University Press, New York, U. S. Agents). Price, $2.75.

This volume is No. 2 of the Memoirs of the Society for Endocrinology and contains nine papers concerned with techniques and methods used in the determination of adrenocortical steroids. The third paper describing the fascinating researches of Simpson and Tait on the trail to what is now recognized as aldosterone, is of particular interest.

Professor Marrian's closing remarks are of interest; in essence that methodology for determining urinary steroids is delayed for three reasons: "(1) there is no satisfactory method for hydrolysis of the steroid conjugates without loss, (2) extraction methods leave much to be desired, and (3) that our knowledge of the chemical nature of the compounds we try to determine is still woefully incomplete."

Unlike No. 1, Memoirs of the Society for Endocrinology, the subject material in these papers is devoted to a fairly well demarcated area thus making the volume more meaningful.

With the rapid expansion in our knowledge of the role of the adrenocortical hormones in the total body economy it becomes necessary for the clinician to have an understanding of the techniques used and difficulties encountered in the determination of adrenocortical steroids. Symposia of this type are of value and are worthy of the time spent in the study of their contents by both the clinician and steroid chemist. B. C.

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*Developmental Concordance and Discordance During Puberty and Early Adolescence.* By Douglas M. More. Pp. 128. Champaign, Ill.: Child Develoment Publications of the Society for Research in Child Development, Inc., 1955.

This monograph is part of a project conducted by the Committee on Human Development of the University of Chicago. It concerns a special statistical relationship between three areas of development (physical, social, and emotional) in "typical" adolescents tested and rated over 7 years. In part, the author finds an abrupt physical change in girls and a more linear one in boys, noting that repression of sexual impulses must be almost complete in girls, while boys' emotional defense is ego restriction. He comments that good social adjustment largely seems to be based upon denial of any real emotional perception of the self and relation to others.

O. E.

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*The Guilty Mind: Psychiatry and the Law of Homicide.* By John Biggs, Jr. Pp. 236. New York: Harcourt, Brace & Co., Inc., 1955. Price, $4.50.

This book contains the third series of lectures to be given under the auspices of the Isaac Ray Award, a series of lectures devoted to clarifying the issues and improving the understanding and cooperation between law and psychiatry. The author begins with primitive man and traces the slow progress of man's effort to deal with the mentally ill criminal through the middle ages, the Renaissance and, finally, to the Twentieth Century. He evaluates some of our humane and scientific efforts to do justice to the criminal as well as to society. Interesting, thought-provoking and highly recommended to the doctor, lawyer and layman concerned in social progress.

O. E.

*An Elementary Textbook of Psychoanalysis.* By Charles Brenner, M.D., Associate Clinical Professor of Psychiatry, Yale University Medical School. Pp. 219. New York: International Universities Press, Inc., 1955. Price, $4.00.

Here is a book explaining the fundamental theories of Psychoanalysis by a lecturer in the New York Psychoanalytic Institute. It is readable, sound, and like many elementary books written by a man who knows his subject, proves to be quite valuable for the person who thinks he knows the subject fairly well but would like a review. The book has eight chapters dealing with the fundamental hypotheses of psychoanalysis, The Psychic Apparatus, Parapraxes and Wit, Dreams and Psychopathology. This book will help the general practitioner understand better the emotional complexes of his patient and will serve as a good orientation text for anyone who would like to acquaint himself with psychoanalytic theory. The book can be recommended to ancillary medical personnel. It contains both a reference reading list and an index. O. E.

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*Second International Congress of the International Diabetes Federation, July 4 to 8, 1955, Cambridge, England.* The Hague, Netherlands: International Diabetes Federation, 1955.

The abstracts of reports presented before the second international forum concerned with diabetes mellitus have been published. The value of this publication could be increased greatly by improving the quality of the abstracts. Physicians interested in the content of the papers would appreciate more informative summaries rather than brief statements concluding with the comment "the significance will be discussed." About 50% of the abstracts are sufficiently detailed to provide the necessary information for those unable to attend the congress. C. S.

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*Biochemical Preparations, Vol. 4.* Edited by W. W. Westerfeld. Pp. 108. New York: John Wiley & Sons, Inc., 1955. Price, $3.75.

The fourth volume of this valuable set is a fine addition to the biochemist's library. It contains methods of preparation of 20 different compounds. They are almost equally divided between synthetic chemical procedures and isolations from biological materials. In this volume are described methods of preparation of two enzymes, five compounds related to carbohydrates, eleven water-soluble nitrogenous compounds and two fatty materials.

A valuable cumulative subject index for the four volumes completes this book. D. W.

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*Differential Diagnosis of Leukoplakia, Leukokeratosis and Cancer in the Mouth.* By Ashton L. Welsh, M.S., M.D., Assistant Professor of Dermatology and Syphilology, University of Cincinnati College of Medicine. Pp. 62. Springfield, Ill.: Charles C Thomas, 1955. Price, $2.50.

This monograph describes the neoplastic lesions of the oral mucous membranes. The author considers leukoplakia and the more advanced form, leukokeratosis to be definitely precancerous. The picturesque descriptions go far to compensate for the absence of illustrations. A full chapter is devoted to describing and differentiating other oral lesions. The author re-emphasizes a tenet which cannot be too often repeated, "any lesion which does not respond to conventional therapy in two weeks, receives microscopic evaluation—promptly." S. L.

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*Skin Diseases: Eczema, Dermatitis, Psoriasis, Erysipelas, Impetigo, Alopecia, Warts, Rashes, and Others.* By Reginald T. Brain, M.D. Modern Health Series. Chicago: Henry Regnery Co., 1955. Price, $2.25.

Dr. Brain has produced a well-written, common sense exposition of skin diseases for the general public. It is full of sound data and will not lead to self-treatment. It contains discussions of the common cutaneous disorders and infections: dermatitis, eczema, psoriasis, warts, eruptions, nevi and other birthmarks, disorders of the scalp and nails, and diseases of the skin in children. The descriptions are accompanied by well-chosen pictures and line sketches which are for the most part well-placed in the text. This volume will be of value not only to the public but will serve as a model for nurses and physicians who must advise patients suffering from affections of the skin. H. B.

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*A New Classification of Tuberculosis with New Diagnostic Standards.* By Milosh Sekulich, M.D. Pp. 63; 11 ills. London: William Heinemann, Medical Books, Ltd., 1955. Price, 3s. 6d.

The author, dissatisfied with the accepted classifications of tuberculosis, proposes one based on his concept of the natural history of the disease. The basic classification is divided into two types: I. Primary, subdivided into inflammatory (benign) and caseous (malignant); and II. Secondary, which includes a fibro-caseous form, and a fibrous form. Further subdivisions, estimates of extent and activity of the disease complicate the basic formula. The index includes 30 variations of pulmonary tuberculosis, 9 epidemiologic divisions, and 23 nonpulmonary forms of the disease.

It is unlikely that the proposed classification will replace the official National Tuberculosis Association classification which, while imperfect, is relatively simple and workable. R. C.

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*An Introduction to Psychiatry.* By Max Valentine, M.D., D.P.M. Pp. 298; illustrated. Edinburgh and London: E. & S. Living-

stone Ltd., 1955 (Williams & Wilkins Co., Baltimore, U. S. Agents). Price, $3.75.

THIS is one of those books written by what might be called an "individual psychiatrist." It presents the author's own concepts and nomenclature for psychiatric disorders such as the terms "Histogenic" and Chemogenic." This may be satisfactory in his own country (Scotland) or for his own medical students but it can scarcely be recommended in this country where textbooks on psychiatry have tended to follow either the psychobiological orientation of Meyer or the psychoanalytical orientation of Freud. Moreover, the book is small and the brevity with which each of a large number of subjects is covered is disadvantageous. An appendix gives a typical case presentation of work of psychiatric entities but treatment formulation is lacking.

O. E.

---

*British Encyclopaedia of Medical Practice Including Medicine, Surgery, Obstetrics, Gynaecology and Other Special Subjects. Medical Progress, 1955.* Pp. 324. London: Butterworth & Co., 1955.

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*Biologisch-Medizinisches Taschenjahrbuch, 1956.* By DR. HANS HAFERKAMP. Pp. 662. Stuttgart: Hippokrates-Verlag, 1955. Price, DM 5.

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*The Diagnosis and Treatment of Haemophilia and Its Related Conditions.* By R. G. MACFARLANE, M.D., and ROSEMARY BIGGS, M.D. Pp. 23; 2 ills. London: Her Majesty's Stationery Office, 1955. Price, 2s. 6d.

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*Respiratory Problems in the Premature Infant.* M & R Pediatric Research Conference. Pp. 87; 13 ills. Columbus, Ohio: M & R Laboratories, 1955.

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*Nutrition in Infections.* By W. A. WRIGHT and 26 other investigators. Ann. New York Acad. Sci., Vol. 63, Art. 2, pp. 145–318. Pp. 174; illustrated. New York: New York Academy of Sciences, 1955. Price, $4.00.

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*Dried BCG Vaccine.* World Health Organization Monograph Series No. 28. By YOJI OBAYASHI. Pp. 220; 57 ills. Geneva: World Health Organization, 1955. Price, $5.00.

THIS monograph contains a well organized body of information, heretofore available chiefly in Japanese publications. The Research Institute of the Japan Anti-Tuberculosis Association has been engaged in the mass production of dried BCG vaccine since 1947. Production methods, and many experiments testing the safety, potency, viability and preservability of vaccine are described in detail. Admitting that methods can be further improved, the author believes that dried BCG vaccine, produced by the methods described, is more convenient and effective than liquid vaccines. This would appear to be especially true for mass vaccination programs in tropical and semitropical climates.

R. C.

---

*Psychosomatics.* By MAX HAMILTON, M.D., D.P.M., Senior Lecturer, Department of Psychiatry, University of Leeds School of Medicine. Pp. 225; 16 ills. New York: John Wiley & Sons Inc., 1955. Price, $4.25.

THIS is a special type of book on psychosomatic medicine. It covers the theories and philosophies of psychosomatic medicine. It includes many of the outstanding contributions to this field. It summarizes many of the pertinent findings over the past 25 years. It gives the psychological structure, the psychopathology of, for instance, the patient suffering with gastrointestinal disorders, with asthma, or rheumatism. It lacks clinical case material and treatment directions and this constitutes an omission of considerable moment in a field where psychiatric techniques are so important. But the book gives an excellent orientation and summary of the philosophical approach to psychosomatic disease and summarizes so well the contributions that have been made that it deserves recommendation as a compendium of psychosomatic disease.

O. E.

---

*Experimental Pharmacodynamics.* By T. KOPPANYI, Georgetown University Medical School, Washington, D. C., and A. G. KARCZMAR, Sterling-Winthrop Research Institute, Rensselaer, New York. Pp. 256; 31 ills. Minneapolis: Burgess Publishing Co., 1955. Price, $5.00.

THIS laboratory manual is suitable for a course in pharmacology for medical students. It includes well-chosen workable experiments, mainly utilizing the common laboratory animals. The experiments cover important pharmacological principles and the chief agents used in medicine. The experiments on each group of drugs are preceded by a brief discussion of important pharmacological considerations. Following each group of agents there is a section entitled "Clinical Bearings," in which the actions of the drugs are incisively related to their clinical use and in which the present status of the agents in therapy is critically appraised. There are useful bibliographies at the end of each chapter. There is a concise appendix of 12 pages on prescription writing. This manual has evidently been developed over many years with care and insight, and has been kept abreast of the rapid developments in pharmacology. It is refreshing to see so much effort lavished on the education of medical students. This manual should be highly acceptable to the teacher who does not wish to choose and devise his own laboratory experiments.

D. C.

*To Define True Madness: Common Sense Psychiatry for Lay People.* By Henry Yellowlees. Pp. 172. Paper. Pelican Books, A 357. Baltimore: Penguin Books, Inc., 1955. Price, 65 cents.

Any attempt "to give non-medical readers a fuller and clearer understanding of . . . disorder with which psychiatry deals" necessarily enters the highly controversial field of combat between the a-little-knowledge-is-a-dangerous-thing-ers and those who attempt to relieve, even if in the slightest degree, the ignorance of an interested public in some important field. The author, a British psychiatrist of distinction who obviously belongs in the latter class, presents from the British point of view several neuropsychiatric manifestations with comment on desirable methods of treatment.

In the early pages of the book, however, there appear radical differences in the British public's attitude toward psychiatry, and in the author's too for that matter, from those held in this country. These so weaken the presentation to American readers that it seems doubtful if such a book should have been included in a Pelican series in this country.

Exception must also be taken to the chief title, "To Define True Madness," which is not only less appropriate than the secondary title but is actually misleading. The reviewer regrets the time wasted in hunting for the title's justification. Only the one chapter, "Guilty but Insane," deals primarily with insanity; all the others are concerned with explanations of mental processes in general and with the neuroses, under such headings as depression, inferiority complex and anxiety neurosis. One wonders whether perhaps this discrepancy between title and text may be due to an ill-considered use of the scissors on a much larger text.

As one of the lay readers, psychiatrically speaking, for whom this book was intended, this reviewer regrets that such a popular and useful group as the Pelican Series should have included it in the American distribution of Penguin books, notwithstanding the evidences of the author's common sense that are scattered through the booklet and his popularity with the British public.

E. K.

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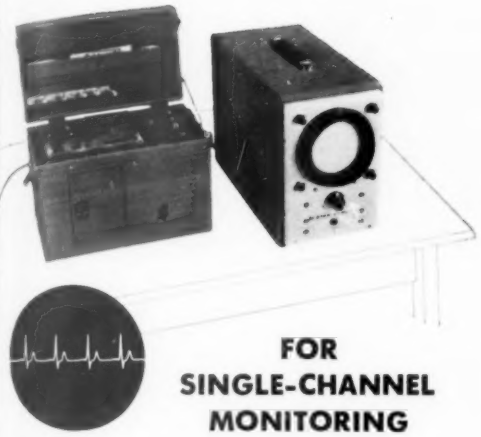

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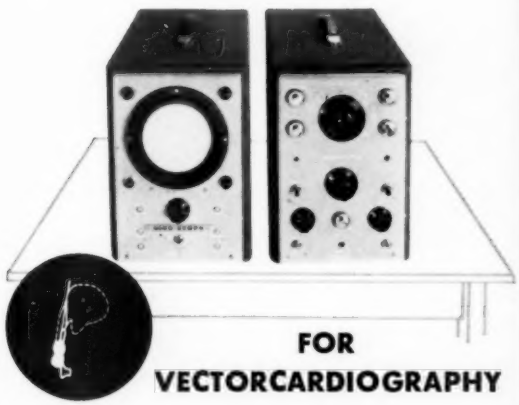

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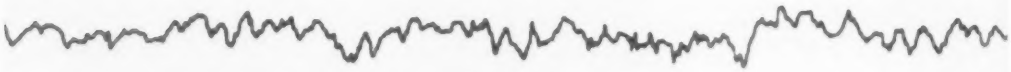

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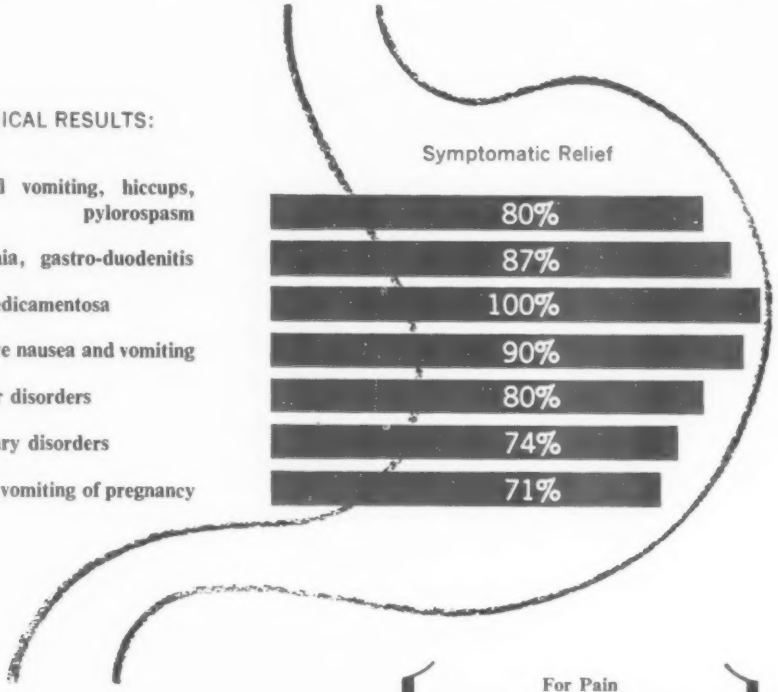


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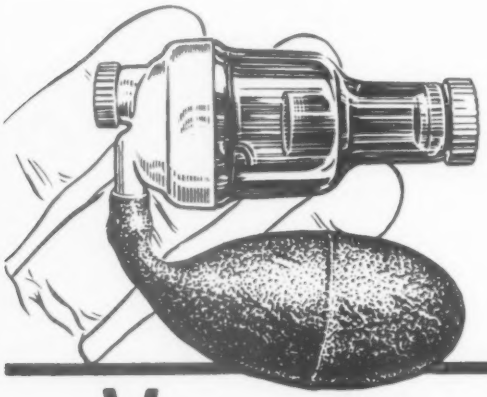

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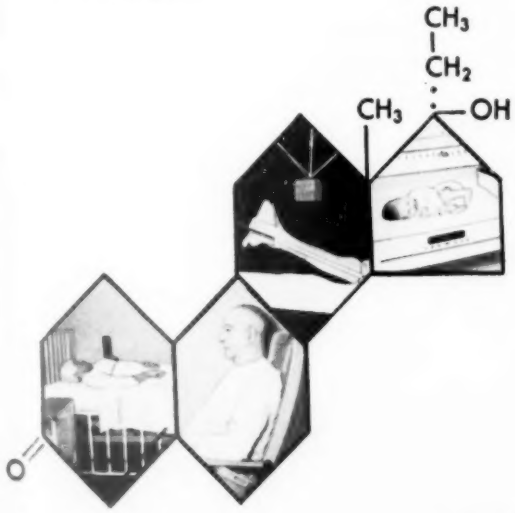


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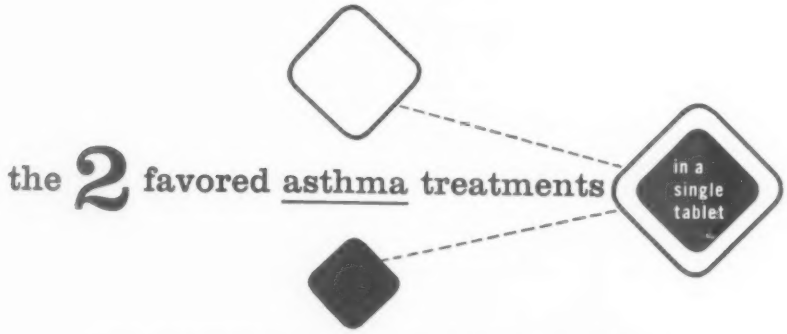


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| Aluminum hydroxide gel, dried . | 0.12 Gm. |
| Calcium ascorbate . . . . . | 60 mg. |
| (equivalent to 50 mg. ascorbic acid) | |
| Calcium carbonate . . . . . | 60 mg. |

* U.S. Pat. 2,691,662

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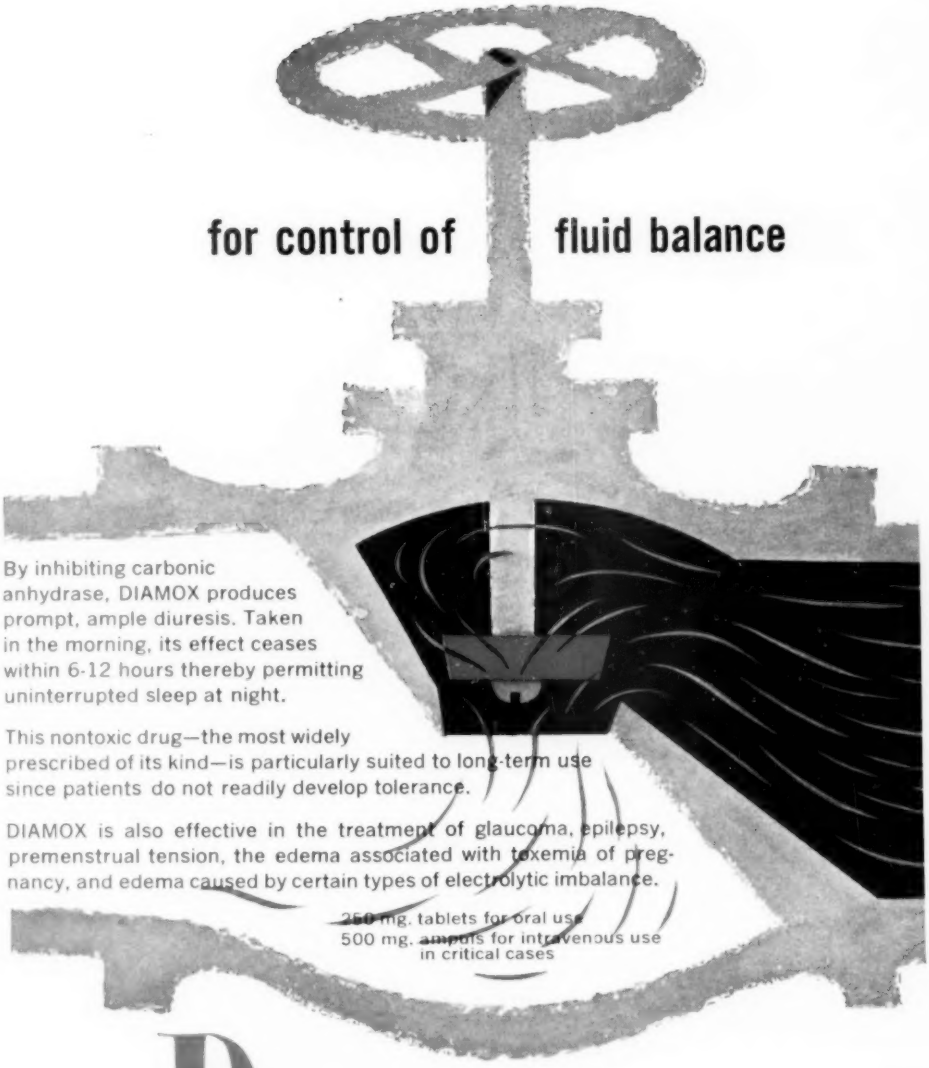

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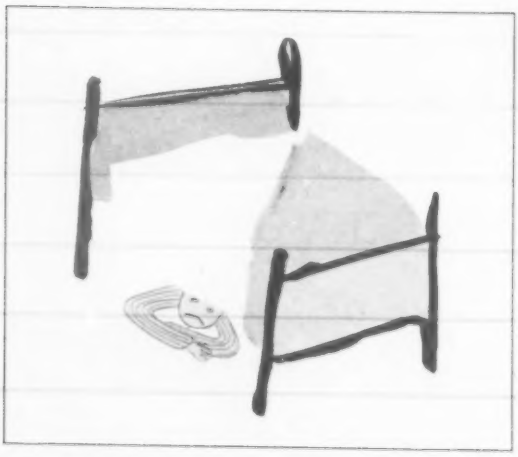

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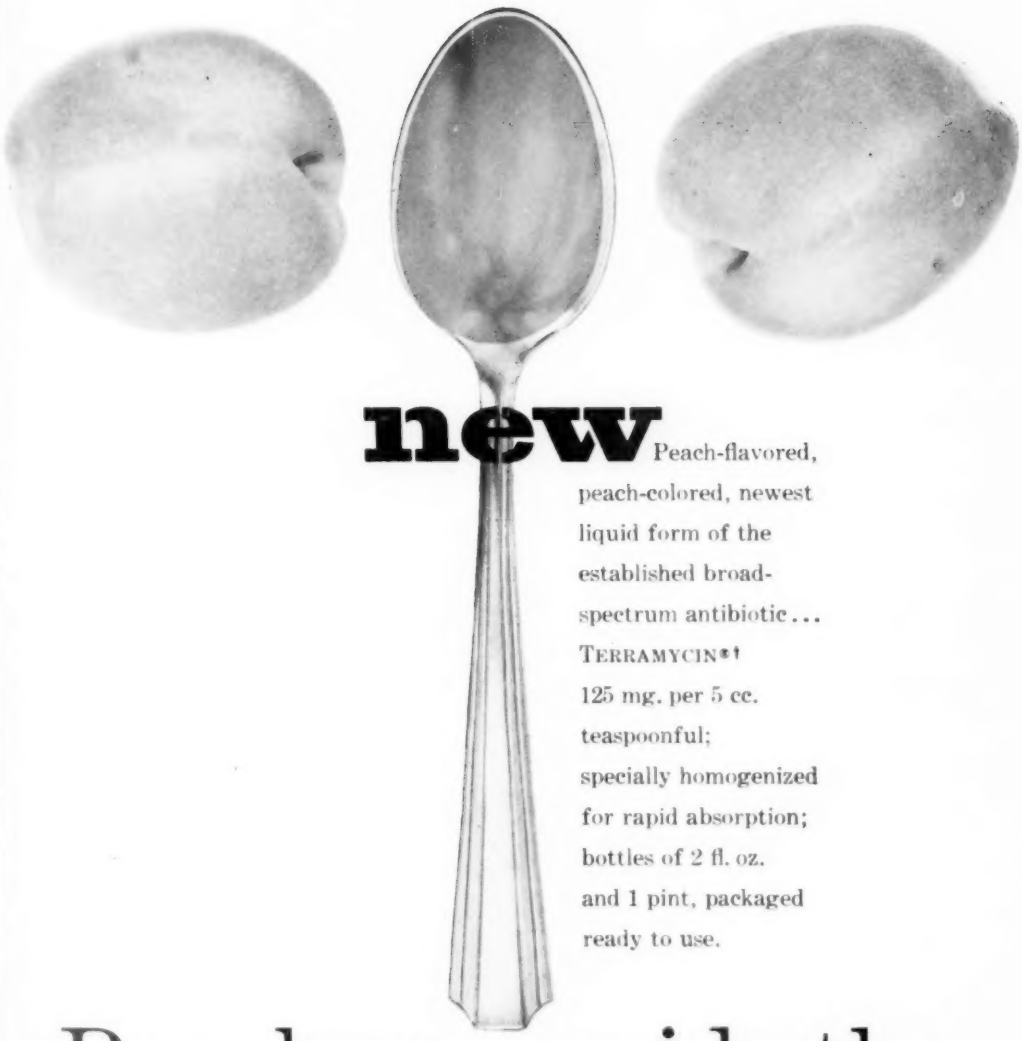

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*Reifenstein, E. C., Jr., in Harrison, T. R.: Principles of Internal Medicine, ed. 2, New York, The Blakiston Company, Inc., 1954, chap. 98, pp. 702, 703.

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References: 1. Ehrlich, J. C.: Arizona Med. *12*: 239 (June) 1955. 2. Weiss, A., and Steigmann, F.: Am. J. M. Sc. *227*: 188 (Feb.) 1954. 3. Dimitroff, S. P.; Griffith, G. C.; Thorner, M. C., and Walker, J.: Ann. Int. Med. *39*: 1189 (Dec.) 1953. 4. Hejtmancik, M. R., and Herrmann, G. R.: Texas St. J. M. *51*: 238 (May) 1955. 5. Batterman, R. C.; DeGraff, A. C., and Rose, O. A.: Circulation *5*: 201 (Feb.) 1952. 6. Denham, R. M.: J. Kentucky St. M. Assoc. *53*: 209 (Mar.) 1955.

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- rapid absorption — promptly reduces systolic and diastolic pressures
- consistent and predictable response — smoother control
- lower dosage required than with other ganglionic blockers
- minimal likelihood of drug tolerance

## Clinical observations

In a study of four ganglionic blocking agents, Winsor[1] found that the "most effective agent was SU3088 [Ecolid]...." In another comparative study, Grimson[2] reported: "Results with Ecolid have been definitely more encouraging than those with pentolinium." Patients maintained on Ecolid state that they prefer this ganglionic blocking agent because of greater energy, improved appetite, less difficulty with constipation and fewer tablets to take.[2,3]

*For complete information about Ecolid, particularly more details on dosage recommendations, management of undesired effects and precautions, contact your CIBA representative or write to Medical Service Division for booklet entitled "Ecolid — A New Ganglionic Blocker for Hypertension."*

*References:*

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SUPPLIED: ECOLID Tablets (Rotocotes), 25 mg. (ivory) and 50 mg. (pink).

DOSAGE: Dosage must be adjusted to the individual patient. Below is a typical plan by which treatment may be initiated.

| Ambulatory patients | | |
|---|---|---|
| DAY | A.M. | P.M. |
| 1 | 25 mg. | — |
| 2 | 25 mg. | 25 mg. |
| 3 | 50 mg. | 25 mg. |
| 4 | 50 mg. | 50 mg. |
| 5 | 75 mg. | 50 mg. |
| 6 | 75 mg. | 75 mg. |
| 7 | 100 mg. | 75 mg. |
| 8 | 100 mg. | 100 mg. |

| Hospitalized patients | | |
|---|---|---|
| DAY | A.M. | P.M. |
| 1 | 50 mg. | — |
| 2 | 50 mg. | 50 mg. |
| 3 | 100 mg. | 50 mg. |
| 4 | 100 mg. | 100 mg. |

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# REPRESENTATIVE CLINICAL STUDIES OF Ecolid*

| Number of Patients | Initial Oral Dosage | Responses | Duration of Action | References |
|---|---|---|---|---|
| 59 (Total Series) | 75 to 300 mg. daily | Compared with other ganglionic blockers, small doses of Ecolid were employed and greater hypotensive effect was obtained. Rapid absorption and long duration of hypotensive action. | Postural hypotension lasted 13.4 hours in 5 "test" patients receiving doses of 150 mg. | 1 |
| 20 | 50 to 200 mg. daily | Blood pressure in 20 well controlled; reductions lasted twice as long as those induced by pentolinium. Each of 10 patients with previous experience with hexamethonium preferred Ecolid. Less difficulty with constipation; appetite improved; greater energy. | ** | 2 |
| 18 | 50 to 100 mg. daily | Hypertension in 18 well controlled. Supine blood pressure reduced without tachycardia. Constipation occurred infrequently. | Supine blood pressure lowered for 12 hours or more with single oral doses of 50 to 100 mg. | 3,4 |
| 44 | 50 mg. daily | 35 responded well; 14 of these became normotensive. All patients received reserpine as base therapy. | ** | 5 |
| 12 | 25 to 200 mg. daily | Blood pressure of all 12 satisfactorily controlled. Systolic blood pressure lowered average of 76 mm. Diastolic blood pressure lowered average of 42 mm. | ** | 6 |

*To date, a total of 63 investigators have reported on the use of Ecolid in more than 500 patients. They were practically unanimous in the opinion that Ecolid was highly effective. Nearly all commented on the prolonged duration of action—about 8 to 12 hours—which permitted a twice daily dosage schedule in most cases.

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[1]Albertson, H. A. and Trout, H. H., Jr.; *Antibiotics Annual* 1954-55, Medical Encyclopedia, Inc., New York, N. Y., 1955, pp. 599-602.

[2]Prigot, A.; Whitaker, J. C.; Shidlovsky, B. A., and Marmell, M.: *ibid*, pp. 603-607.

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# Spatial Vectorcardiography

By GEORGE E. BURCH, M.D., F.A.C.P.

*Henderson Professor of Medicine, Tulane University School of Medicine; Physician-in-Chief, Tulane Unit, Charity Hospital of Louisiana at New Orleans; Consultant in Cardiovascular Diseases, Ochsner Clinic, etc.*

J. A. ABILDSKOV, M.D.

*Instructor in Medicine, Tulane University School of Medicine, Assistant Visiting Physician, Tulane Unit, Charity Hospital of Louisiana at New Orleans*

and JAMES A. CRONVICH, M.S.

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THE material in this useful book is based on a very successful scientific exhibit recently established and conducted by the authors. It is an enlightening summary of the principle of vectorcardiography and presents, in clear, simple terms, the meaning of spatial forces and the concept of spatial vectorcardiography.

The authors have written this book especially for those not acquainted with the science of vectorcardiography, but who realize the importance of this relatively new field in electrocardiography. They present their own experimental results and opinions concerning the role of vectorcardiography in clinical medicine, as well as in experimental cardiology and electrocardiography. Vectorcardiography is correlated with electrocardiography when it is deemed necessary or advisable.

**VECTORCARDIOGRAM: A record of the time course of the mean instantaneous electric spatial vector forces of the heart.**

**SPATIAL VECTORCARDIOGRAM: Since the heart is a three-dimensional structure located in a volume conductor (the body), the electric forces associated with the heart beat have *direction and magnitude in space*. The vectors shown in this book are actually spatially located, and the vectorcardiogram is oriented in three dimensions.**

Principles and methods of recording in spatial vectorcardiography are given in the first part of the manual. This information is a sound aid to those not familiar with the subject. Materials and methods used in studies made in the authors' laboratory are described briefly and followed by individual chapters on the use of spatial vectorcardiograms in specific conditions.

*173 Pages.* *121 Illustrations.* *$5.00*

(Published October, 1953)

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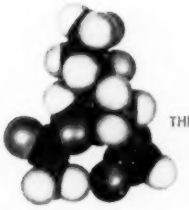

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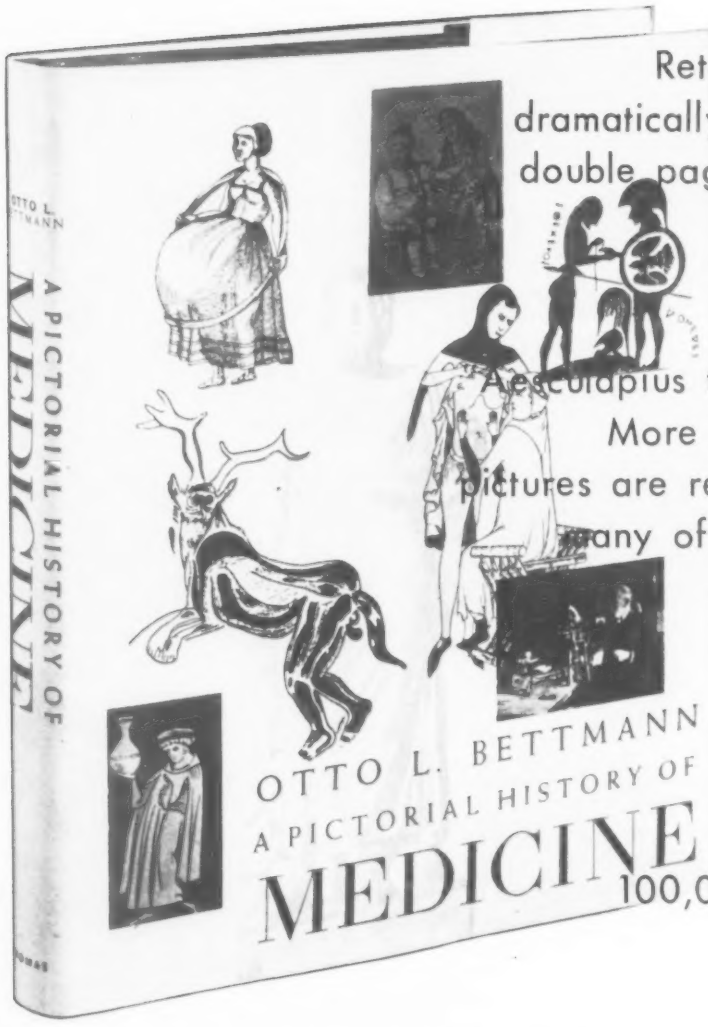


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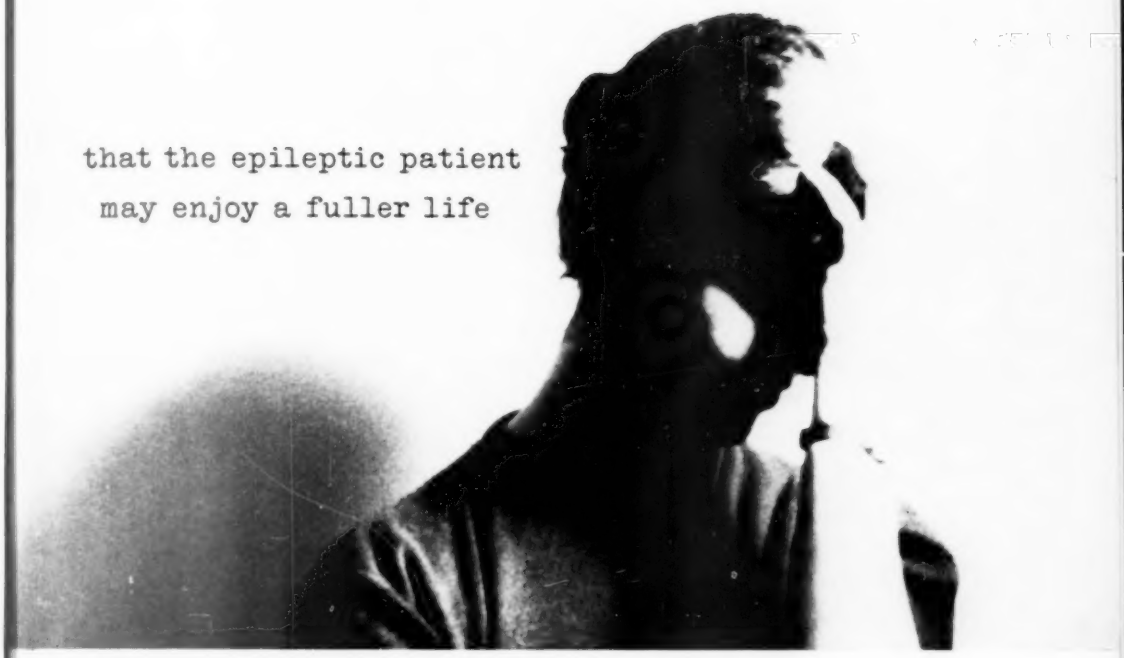

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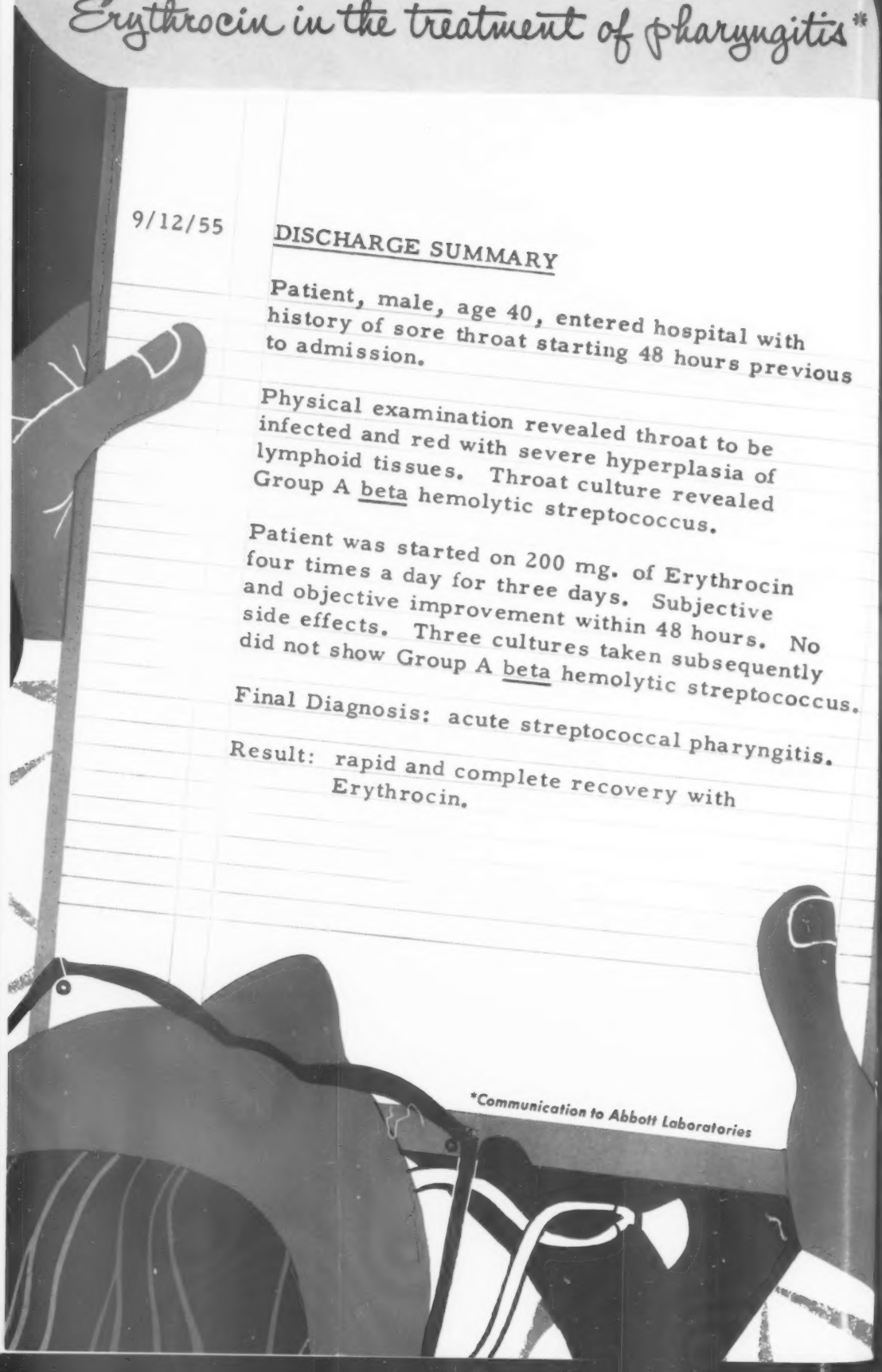

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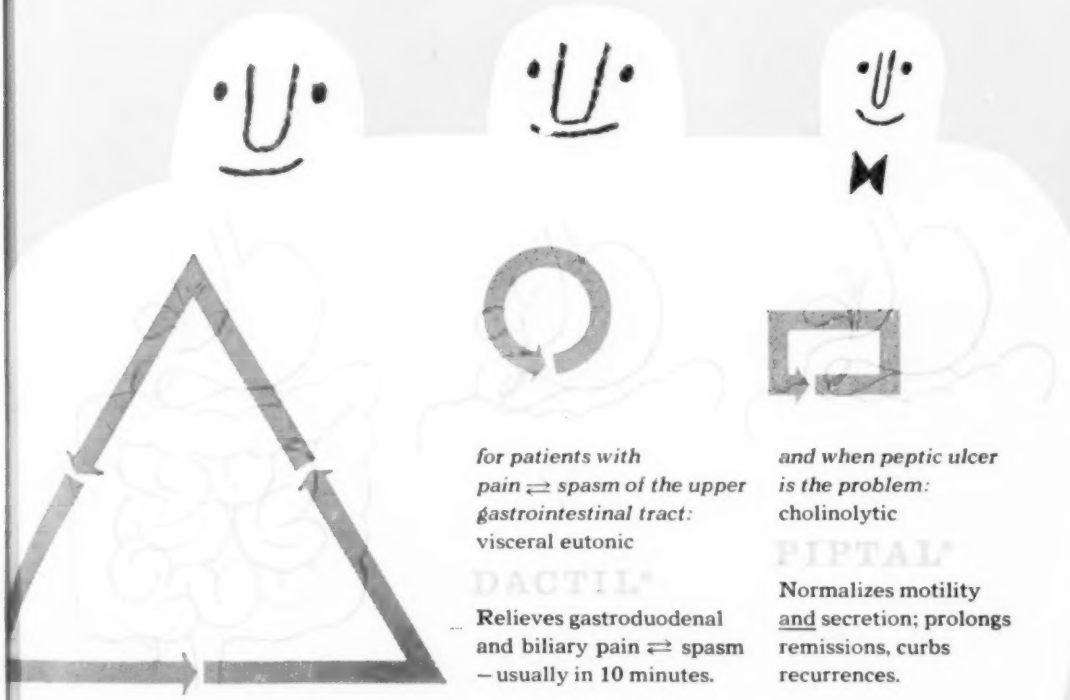

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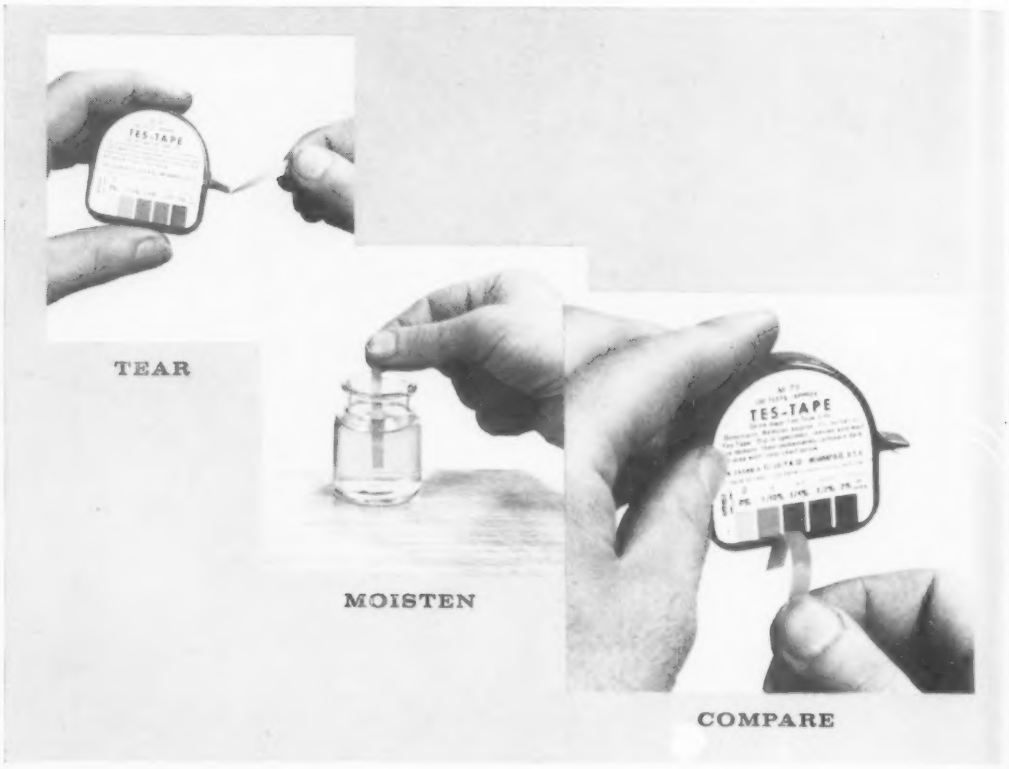


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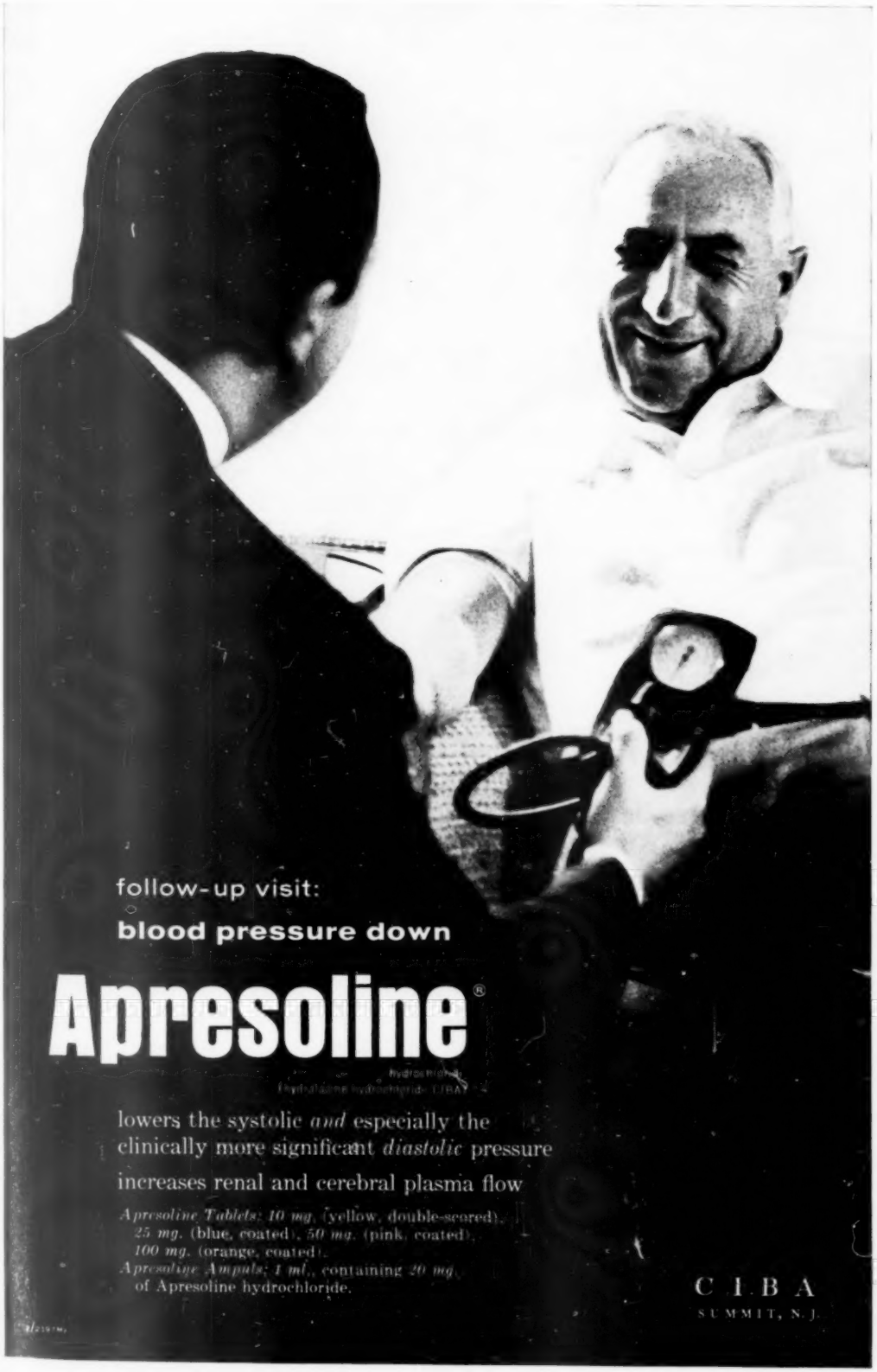
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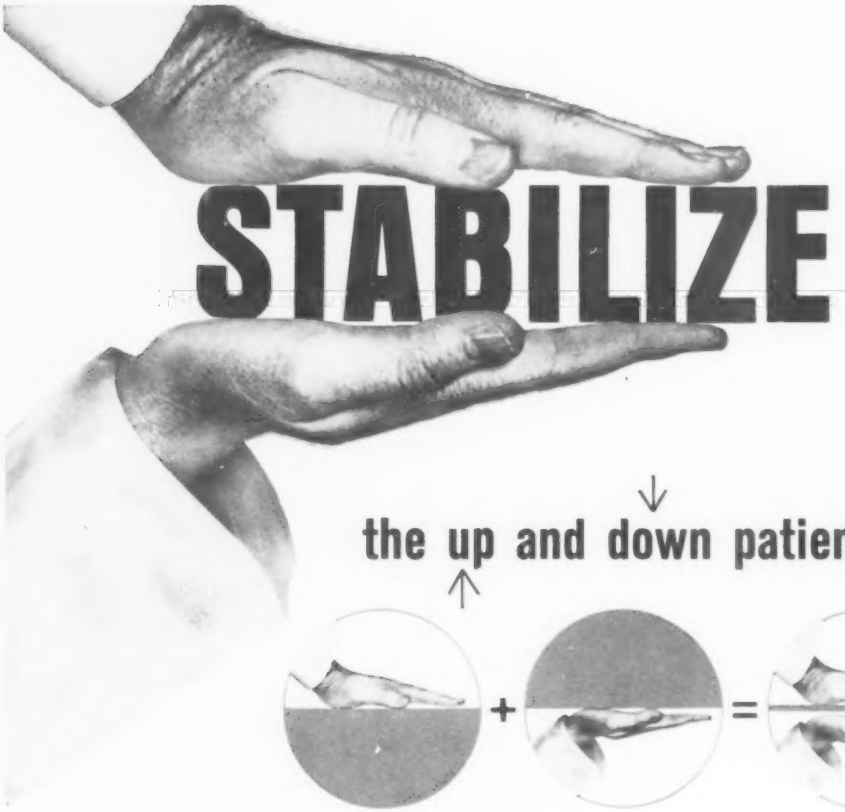

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